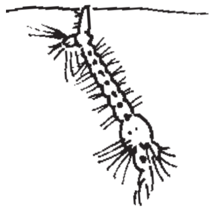
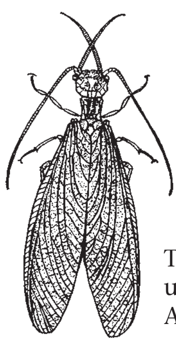


REFINEMENT AND EXPANSION OF WETLAND BIOLOGICAL INDICES FOR WISCONSIN

Final Report to the U.S. Environmental Protection Agency Region V

Richard A. Lillie, Paul Garrison, Stanley I. Dodson,
Richard A. Bautz and Gina LaLiberte

APRIL 2002



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Final Report to USEPA - Region V
Wetland Grant #CD975115-01-0

April 2002

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EXECUTIVE SUMMARY

Six separate wetland biological communities, namely macroinvertebrates (primarily comprised of aquatic insects, snails, and other macro-crustaceans), microinvertebrates (i.e., zooplankton), diatoms (a type of algae found in all waters), amphibians (frogs and toads), small mammals (shrews, voles, and mice), and wetland plants, were evaluated as possible indices of wetland ecological integrity or health. Selected metrics of each community, except within the small mammal community (which probably failed because there were too few species to provide a useful index), were clearly related to a surrogate measure of human disturbance and a priori classification.

This report summarizes the methodology employed in monitoring each community and identifies the metrics (i.e., community attributes such as taxonomic richness that respond significantly to disturbance) incorporated into five biological indices. The performance of each index was evaluated by determining its ability to distinguish statistically significant differences among sub-sets of wetlands categorized a priori as to watershed land use type (e.g., agriculture versus urban versus natural land use), sub-classes (e.g., industrial versus residential urban lane use), agricultural intensity (3 categories based on percent agricultural development), and buffer width class (narrow versus wide cover in wetland perimeter), and a posteriori classification among five objective-based impact classes (impact based on chloride and total nutrient concentrations).

Each of the five biotic indices was largely successful in separating impacted wetlands from least-impacted reference wetlands. None of the indices was able to separate effects among the levels of agricultural intensity, and only the diatom index was able to separate between narrow and wide buffer classes.

A composite index incorporating the five biotic indices was developed to represent an Index of Ecological Integrity for Wisconsin long duration wetlands. While the Index of Ecological Integrity performs better than the component biotic indices, it is recommended that any of the five indices may be applied separately to evaluate the current condition of Wisconsin wetlands.

Continuing research and field application of the methods presented here are being implemented by the Wisconsin Department of Natural Resources.

INTRODUCTION

This study builds upon work conducted under a previous USEPA Wetland Grant (#CD985491-01-0) that funded the preliminary development of a biological index and classification system for Wisconsin wetlands using macroinvertebrate and plant communities (see Lillie 2000). The basic objective of the original study (1998-1999) was to develop a hierarchical approach to classify and rank wetlands as to their biological purpose, condition, and relative rarity.

Background:

In the past decade, a considerable effort has been made at local, state, and federal levels to protect, restore, and create wetland habitat. This effort was based on the knowledge that wetlands perform a myriad of functions to mankind; functions which include physical, chemical, and biological components. The relative significance of these functions differs according to the spatial position of individual wetlands in the landscape or the temporal stage of the wetland with respect to dynamic climatic cycles. The biological function of wetlands, while obvious in general context to most biologists or ecologists, is frequently questioned by developers, engineers, and the public. This is especially true when those wetlands are small, transient or temporary, or hinder man's attempts to farm, build roads or other structures, or where the wetlands simply appear to serve as breeding areas for hoards of nuisance mosquitoes. The biological function of wetlands is addressed in Wisconsin's Wetland Water Quality Standards (Wisconsin Administrative Code NR 103.03(1)(e-f)), which provides for the protection of habitat for aquatic organisms and resident and transient wildlife, and in NR 103.03(2)(e), which protects the hydrological conditions necessary to support these biota. Due to the complexity of hydrological and ecological conditions associated with the many different types of wetlands found in Wisconsin, the code was established with simple narrative water quality criteria or conditions rather than specific numerical criteria. The intent of this study is to provide the tools, in this case a suite of biological indices and a classification system, that may be used to quantify, characterize, rank, and define biological function and ecosystem integrity of wetlands.

The justification for the development of biological-based indices for monitoring the quality or health of wetlands is quite simple. Biomonitoring is an efficient means to measure the integrated impacts of both human and natural forces on the entire wetland ecosystem operating over a variable temporal scale. Whether induced by sudden discrete events (e.g., chemical spills), pulses (seasonal acidic inputs accompanying snow-melt runoff), or longer term climatic induced changes, the flora and fauna of wetlands respond to environmental change in different fashion. The adaptive capacities of the individual species and their responses vary according to the strength (dose), duration, and character of the disturbance. Changes in species composition can and do occur, with some species eventually being extripated from a site. Such changes might not be detected if only chemical sampling were used to monitor the health of the wetland. Responses among the biota may represent identifiable signatures that convey specific information as to the causative agent or force acting upon the community in question. Biological resilience and stability of a community are weakened by repeated disturbance, and the overall biological health of a wetland community reflects the historical exposure of multiple stressors.

In this respect the biological community is clearly superior to chemical sampling as a means of determining the impact of pollutants to wetland ecosystems.

In a preliminary investigation (Lillie 2000), we explored the utility of using multimetric indices based on macroinvertebrate (primarily aquatic insects) and plant community attributes (or more correctly, attributes that are responsive to human impacts – i.e., metrics). Basically, the wetland indices were intended to serve as tools to quantify, characterize, rank, and define biological function and ecosystem integrity of Wisconsin wetlands. In the current study, we proposed to (1) evaluate the performance of the two preliminary indices on an independent set of wetlands and (2) revise those indices as necessary to maximize their discriminatory power, and (3) to expand index development to include an additional suite of four biological communities. Namely, the four groups are diatoms, zooplankton, amphibians, and small mammals. Adding new assemblages (and evaluating each independently one from the other) allows the opportunity to increase the ability to identify stressors (Danielson 1998) and ultimately provide a more accurate assessment of true ecological integrity. Development of a combined index based on an expanded group of assemblages will allow assessment of the ecological integrity of Wisconsin's numerous depressional, palustrine, seasonal and semi-permanent wetlands of aquatic bed, emergent, and forested classes (classes of Cowardin et al. 1979).

Each of the four additional biological communities provides a somewhat different perspective of the ecological condition of a wetland ecosystem. Diatoms offer the advantage of measuring both current (surface sediment layers) and historic (core profiles) conditions within individual wetlands over time. Diatoms have been used extensively both in Wisconsin (Kingston et al., 1990; Garrison and Winkelman, 1996; Marshal et al., 1996; Garrison and Wakeman, In review,) and elsewhere to evaluate changes in pH and trophic condition in lakes (Bennion et al. 1996, Reid et al. 1995). Other states have incorporated or are considering incorporating algae metrics into their wetland indices (Danielson 1998). Zooplankton are extremely sensitive to toxic compounds and consequently offer insight into local transport and impact of pesticide runoff. Amphibians serve as integrative indicators of both the condition of the wetland proper and its surrounding riparian environment inasmuch as they depend upon both habitats for their survival (Lehtinen et al. 1999). Utilization of wetland habitats by small, primarily terrestrial mammals (e.g., shrews and mice) reflect the quality of wetlands and consequently also exhibit promise as indicators of overall wetland health. Small mammals are extremely sensitive to changes in vegetative structure.

The expanded index of ecological integrity will serve as a multipurpose tool to assess current condition, monitor trends, evaluate wetland restoration efforts, aid in future wetland acquisitions, and establish biocriteria for wetlands. This proposal was developed and submitted in response to the Wisconsin Department of Natural Resource's Wetland Team objectives and recommendations for the Division of Waters work planning which calls for the development of new tools to assess and monitor wetland ecological health.

METHODS

Wetland Study Sites:

The original study design for the project called for sampling approximately 80 wetlands meeting the following criteria. All wetlands were to be small, with surface acreage less than 4 acres. Wetlands were to be depressional, paulustrine, not interconnected with adjacent basins containing resident fish communities. Approximately 30 wetlands were to represent relatively undisturbed or least-disturbed reference wetlands, and another 40-50 wetlands would represent a continuum or gradient of disturbed conditions resulting from a combination of urban and agricultural impacts. Within the set of agriculturally impacted wetlands, we had intended to find an equal number of basins in each of six combinations representing three different classes of agriculture land use (light, moderate, and high) and two classes of protective buffer widths (e.g., narrow and wide). We intended to include 10-14 urban wetlands in the study. Wetlands were to represent temporary, seasonal, or semi-permanent wetlands of aquatic bed, emergent, or forested classes (of Cowardin et al. 1979), and basins were preferably to be located on public lands. All wetlands were to be located in the Southeast Wisconsin Till Plains ecoregion (Omernik & Gallant 1988). These restrictions were intended to minimize variability and assure adequate sample sizes within wetland classes.

In accordance with these criteria, a list of over one hundred candidate wetlands was prepared in a cooperative effort with individuals representing professional wetland management and science staff from county, state, federal agencies and private and public universities, institutions, and non-profit organizations from across southern Wisconsin. Two unforeseen factors arose that interfered with sampling plans and dictated changes in sampling design. First, a major drought occurred, which resulted in the total desiccation of all temporary, seasonal, and most semi-permanent basins in southern Wisconsin. The drought began in the summer of 1999 and ended after the initial April-May 2000 sampling period. The drought prohibited sampling basins with short to moderate water duration as originally intended. Consequently, wetland selection was restricted to semi-permanent and permanent basins only.

A more serious problem was that created by the general lack of cooperation by private landowners in Wisconsin. The search for potential study wetlands necessarily included wetlands on private lands, particularly those in agricultural settings, to fulfill the allotment of impacted sites. However, access was often denied when we announced to the landowner that we were employed by the WDNR. Despite our reassurances otherwise, many landowners declined to participate in the study for fear that our agency might 'use' the information derived from the study in legal actions against them.

Consequently, we modified the allotments among impacted and reference wetlands as shown in Table 1, below. The final selections included 38 impacted sites and 36 least-impacted reference sites. The impacted sites included 18 urban sites and 20 agriculture sites. The a priori classification of these 38 sites as being 'impacted' was based on subjective estimation of field biologists using current land use (within the entire surface drainage watershed) and riparian characteristics, and therefore represented suspected impacts. Water chemistry and biota (wetland flora and fauna) were not incorporated into making the classifications. Therefore, a priori classification did not assure that a wetland was actually 'impacted' nor did classification reflect the history of the site (i.e., a site may have been severely impacted in its past history). Similarly, some sites classed as least-disturbed also may have been recovering from historical impacts. The reference sites included 19 wooded kettles and 17 prairie type wetlands. We expanded the number of urban wetlands sampled to include nine basins within predominantly industrial/commercial watersheds and nine basins in residential watersheds.

The set of agriculturally impacted wetlands was further subdivided into two classes based on the average width of their vegetative buffers as being greater (i.e., wide) or less (i.e., narrow) than 10 meters. Buffers consisted of any form of cover not plowed or pastured, including grass, shrub, and wooded. It should be emphasized that the riparian zone land cover percentages (presented later) do not necessarily represent the 'buffer' cover because the former represents the land cover within the entire 30 meter radius about each wetland. In addition, some wetlands with an average buffer width greater than 10 meters may have had some perimeter edge with as little as zero vegetative buffer (e.g., 50% of wetland perimeter had a vegetative buffer greater than 20 meters width; the other 50% of wetland perimeter had no buffer). The categorization also does not account for the spatial positioning of the buffer; i.e., the position of the buffer in relation to surface slope and runoff was not factored into the design of the study. Consequently, the buffer width may have been wider or narrower than 10 meters on the upslope side of a groundwater flow-through basin, where it would have the most effect in filtering surface runoff.

Agriculturally impacted wetlands were also classed on the basis of the percentage of their entire watersheds being in agricultural production – subjectively determined as low < 50%, moderate 50-75%, and high > 75%. Because many of the agricultural wetlands were of moderately large acreage and situated in depressions of relatively gentle slope, it was not possible to accurately define watershed boundaries during the limited amount of time we had available to us. We made a best guess as to the extent of the surface watershed and the direction of flow for each wetland in making each determination. Therefore, although the categorization of individual wetlands into each class of agricultural intensity (i.e., high, moderate, or low intensity) may not be entirely accurate, all wetlands classed as agriculturally impacted most likely received heavier loadings from agricultural runoff than did the reference wetlands.

The distribution of agriculturally impacted wetlands among the different intensity levels and buffer widths was less than what the original proposal called for (i.e., seven in each slot), but the numbers sampled are adequate to apply two-way ANOVA for statistical analysis of land use and buffer class effects.

Table 1. Breakdown of wetlands sampled by disturbance category.

Disturbance Class	**Subclass**	**Buffer Width***	**Number**	**Codes[a]**
Least-disturbed	Prairie	-	17**	P
	Wooded Kettle	-	19	K
Urban-Impacted	Industrial/commercial Commercial	-	9	Ui
	Residential	-	9***	Ur
Agriculturally-Impacted	< 50% row crops	< 10 meters	2	Aqn
		> 10 meters	4	Aqw
	50-75% row crops	< 10 meters	3	Amn
		> 10 meters	5	Amw
	> 75% row crops	< 10 meters	4	Ahn
		> 10 meters	2	Ahw
SUM =			74	

* refers to the *average* width of the undisturbed vegetative buffer around the perimeter of the wetland.
** one additional prairie wetland was sampled for macroinvertebrates during the first sampling period and later discontinued when it became apparent that it was impacted.
*** one site classed as urban residential site (Z32) also had a substantial amount of commercial development and may be classed as either urban category.
[a] Agricultural wetlands were also combined into sets representing buffer widths as Aw (wide buffer, N = 11) and An (narrow buffer, N = 9) or into sets representing the level of agricultural intensity as Aq (low, N = 6), Am (moderate, N = 8), and Ah (high, N = 6).

The seventy-four sampled wetlands were distributed primarily in the Southeast Wisconsin Till Plains ecoregion (Omernik & Gallant 1988, as further modified by Omernik et al. 2000). Four basins were in the adjacent Driftless Area ecoregion (Fig. 1). Site locations were recorded using global-positioning systems (Garmin® GPS 12 MAP) and recorded in decimal degrees for conversion to state WTM83/91 coordinates and subsequent linkage to existing state resource GIS data bases. Wetlands ranged in size from 0.01 to 6.8 acres and in depth (at the time of sampling) from 25 cm to over 110 cm. All wetlands were classified as depressional, palustrine (Cowardin et al. 1979). Wetlands were a mixture of the aquatic bed, emergent, or forested sub-classes (classes of Cowardin et al. 1979).

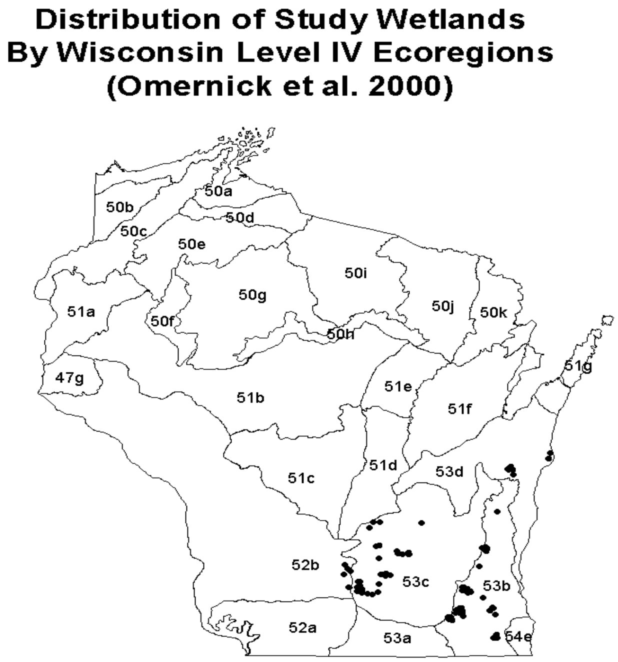


Figure 1.

Sampling Strategy and Schedule:

Environmental conditions, including associated physical, chemical, and biological characteristics of each wetland and its immediate surrounding riparian habitat were measured during field visits in 2000. Four sampling periods were established to coincide with appropriate life cycles of the various biological assemblages (Table 2). The early spring sampling period was established for the macroinvertebrate community to minimize effects of emigration and immigration by flying insects.

Table 2. Schedule for field collection efforts.

Sampling Period Year 2000	Communities sampled
April 17 – May 25*	1st Frog/Toad survey, Macroinvertebrates
May 15 – June 7	2nd Frog/Toad survey, Zooplankton, and water chemistry
July 6 – Aug 1	Plant surveys, Diatoms
Aug 14 – Oct 15	Small mammal trapping

* all but three wetlands sampled before May 10th. B32 dry during earlier visit in April.

Associated Wetland Habitat Attributes:

Field measurements were taken of the following attributes; 1) water temperature - pocket thermometer to nearest 1° C, 2) water depth – meter stick to nearest 1 cm, 3) apparent color and turbidity – visual classification as to stained, turbid, or clear; 4) riparian land use – visual estimate of percent cover of six land use cover types within 30 meters of water's edge, 5) canopy shade cover – visual estimate of percent of wetland surface shaded at noon, 6) types of bottom substrates – visual classification of dominant substrate type, 7) size of wetland inundated – visual estimate of acreage of surface water, and 8) presence or absence of substantial duckweed and algae blooms.

Samples for water chemistry analysis were collected concurrently with zooplankton sampling following standard operating procedures for field collection and preservation as established by the Wisconsin Department of Natural Resources (WDNR). Sample preservatives, lab slips, and labels were provided by the State Laboratory of Hygiene (SLH) in Madison, Wisconsin. Samples were delivered to the SLH within mandated times established by the SLH and USEPA for the following analyses: alkalinity, conductivity, pH, color, calcium, chloride, silica, total Kjeldahl nitrogen (TKN), nitrate and nitrite nitrogen (NO_2-NO_3), and total phosphorus (TP). Total nitrogen was calculated as the sum of TKN and NO_2-NO_3. We collected field replicates from 10% (7 of 74) of all wetlands (randomly selected prior to beginning field work). All laboratory analyses for water chemistry were conducted by the State Laboratory of Hygiene (SLH) using methods approved by the USEPA.

Biological Assessment Methods:

Macroinvertebrates –

Photo 1. Wetland training session in progress, showing D-frame net and seiving bucket used for compositing samples.

Macroinvertebrate communities were assessed using a combination of net sweeps and activity traps. We collected three net sweeps from each wetland using a 12 inch-wide D-frame net equipped with an 800 x 900 micron mesh screen. We employed a standardized sampling strategy in each wetland that consisted of distributing sampling stations evenly about the wetland perimeter to maximize microhabitats sampled and employing a standard net sweep length of 1 meter in water depths less than 60 cm. We rinsed and concentrated contents of net sweeps in the field by examining coarse woody debris for attached macroinvertebrates and saving only the invertebrates and the finer particulate matter for further examination. Sample contents from the three net sweeps were combined to form a single composite sample representative of the entire basin. All samples were preserved in 95% ethanol in labeled 1-quart plastic containers until processed. A set of three activity jar traps (horizontal traps similar in design to that presented by Sherfy et al. 1999) were positioned on marked posts in each wetland (in the same general area as where net sweeps were made) and left in place overnight. Sample contents were retrieved the following morning and preserved in 95% ethanol in labeled sample containers until processed. All nets and jar traps were thoroughly rinsed and examined to remove all organisms before moving them to the next wetland to minimize the chances for cross-contamination of samples. As a means to estimate the variability associated with field collection methods and to answer issues related to representativeness of the data, we collected field replicates from 10% of all wetlands sampled.

Photo 2: Laboratory processing of macroinvertebrate samples.

In the laboratory, macroinvertebrates were sorted and identified at a coarse scale: generally to taxonomic order or family only (e.g., snails, beetles, mosquitoes, fairy shrimp, waterboatmen, etc.). This effort represents taxonomic separations that, with the aid of a simple picture guide, could be made in the field by volunteer staff who are not familiar with invertebrate taxonomy. A complete taxonomic list of aquatic insects found in Wisconsin may be found in Hilsenhoff 1995. Each sample was rinsed on a 500 micron mesh sieve to remove preservative, and the contents were distributed in a shallow tray divided into 24 equal area grids or cells. Beginning with randomly selected cells, all organisms present in each cell were picked, identified, and counted. Abundant organisms, whose total density based on their abundance in the first three randomly selected cells exceeded 300 specimens, were sub-sampled only; their extrapolated abundance was recorded on the data sheet and their presence in the remaining cells was ignored. All other taxa groups were picked, identified, and counted for the balance of the sample. All samples were processed by one individual to eliminate variability created by using different taxonomists. No laboratory replication was possible (due to 100% of sample being processed), and taxonomic verification at this coarse taxonomic resolution was not required. However, voucher specimens were prepared and are available for verification upon request.

Plant Communities –

We assessed plant communities once at each wetland during mid-summer (July 6 to August 1) using a combination of independent methods. The first assessment consisted of a general estimate of total cover by major vegetative type – i.e., percent emergents, submergents, and floating-leafed. Because the three communities often co-occurred, sums of percentages frequently exceed 100%. We also estimated percent wetland area non-vegetated as percent open water.

The second level of plant community assessment consisted of a coarse visual survey of the entire wetland (defined by the edge of waterline) of the general dominance and community composition. This survey was based on a subjective determination of dominance of each plant based on its occurrence (Table P1 below) and was intended to serve as a rapid assessment method requiring a minimum of taxonomic expertise. Plant determinations were made at a very coarse taxonomic level (e.g., cat-tails, sedges, spikerush, pondweeds, etc.). Easily identified taxa (e.g., Reed Canary grass) were identified to species where possible, but in most cases taxa were simply identified as taxon # A, taxon # B, etc. among each of the various genera or families encountered. This coarse level of taxonomy was selected intentionally to permit the application of this methodology by non-botanist field staff (WDNR program managers). Continued sampling and testing of the methodology is currently underway.

Table P1. Subjective classifications of plant dominance based on visual estimates of distribution and cover. Note: explanation of basin coverage may exclude non-vegetated areas.

Classification:	Code:	Description:
"Rare"	(1)	Only 1-3 specimens or clones observed
"Occasional"	(2)	More than 3 specimens or distributions observed, but usually never more than one visible from any single location in the wetland.
"Common"	(3)	Not comprising more than 25% of the total biomass, but usually visible throughout basin (or dense in a restricted portion of the basin).
"Abundant"	(4)	Generally found in large numbers (may be more than 25% total biomass) throughout basin, but other taxa more abundant – not dominant.
"Co-Dominant"	(5)	Generally very important from a standpoint of total biomass or cover, but either subdominant to or shares dominance with other taxa.
"Dominant"	(6)	Extensive coverage and clearly dominant over all other taxa.

The third method was more labor intensive, requiring approximately an hour on each wetland. We measured cover and frequency of occurrence of plants in 18 rectangular quadrats (size of 20 by 50 cm) (per Daubenmire 1959). Quadrats were placed at approximately equal-interval distances (from shoreline to 60 cm depth interval) along three transects (generally laid out in a triangulation of wetland) in the general vicinity of where macroinvertebrates had been sampled in the spring. The absolute cover and frequency of occurrence data were converted to relative cover and frequency values, which were combined to calculate relative importance values (variant of method introduced by Curtis 1947). Importance values were used as attributes in the development of the plant biotic index.

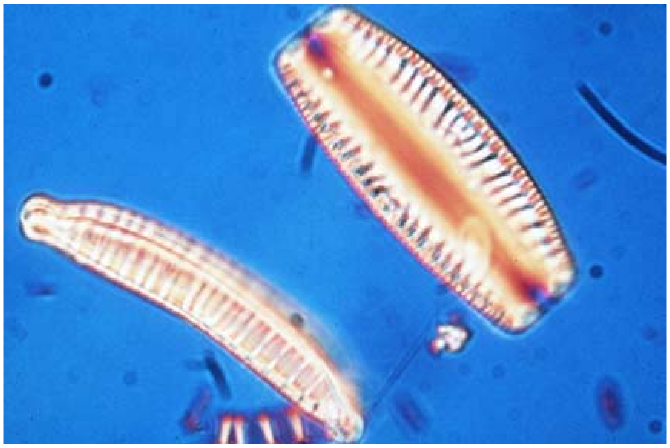
Plant voucher specimens were collected for laboratory verification, and single representative specimens were retained for the Wisconsin Herbarium collection at UW-Madison. Some specimens were retained for the WDNR collection at the Research Center in Monona, WI.

Zooplankton –

We collected a single zooplankton sample from a central basin location in each wetland during the period May 15 to May 25, 2000. We collected surface water using a 5-L plastic bucket, being careful so as not to disturb the bottom or collect large quantities of organic matter, and filtered 3 to 38 L of water through a 200 μm mesh net to capture zooplankton within. Samples were labeled and preserved in 70% ethanol until processed.

In the laboratory, we extracted a series of 1-mL subsamples for quantitative assessments using a Henson-Stemple pipet. As many subsamples were processed as necessary to achieve a minimum of 100 zooplankton. Following the quantitative assessment, we scanned the remainder of the sample to search for species not found in the 100-count method. All specimens were mounted on slides and dissected as necessary to permit species identification and preserved using CMP-10. The total number of Daphnia were counted, with males and females counted separately. Species identifications were made by the principal investigator (Dr. S. Dodson) or, if done by university assistants, checked at random by the principal investigator to control for completeness of the species list.

Diatoms –



We sampled the diatom community in the upper sedimentary layers of each wetland (diatom cell walls are composed of silica, which are preserved in sediments after the organisms die). This sample integrated the diatom community over the last few years. We collected diatom samples during the summer sampling period. Using a one-dram glass vial as a sample collection device, we collected surficial (upper 0-1 cm) sediment samples from five sites in each wetland. The five samples were combined into one composite sample for each wetland. Samples were kept on ice or refrigerated until they were processed. In the laboratory, each sample was thoroughly mixed, and a small amount was placed into a tall beaker. Nitric acid was added and the sample was allowed to steep for about 1 hour. The sample was boiled on a hot plate for 1-2 hours and then neutralized with 30% hydrogen peroxide (M. Julius, pers. comm.). The sample was washed at least four times with deionized water by centrifuging for 10 minutes or by adding deionized water, allowing the cleaned diatoms to settle overnight, and then decanting. One to three drops of the cleaned sample were resuspended with 2 drops 1% HCl in 20 mL of deionized water and pipetted onto a No. 1 cover-slip to dry. The cover slip was mounted with Naphrax® and labeled accordingly. Specimens were identified and counted under oil immersion objective (1400X) until a minimum of 300 and maximum of 500 valves were counted. For all but five of the samples and one replicate, 500 valves were counted. Undetermined specimens representing a significant portion of a sample were sent to Dr. Rex Lowe at Bowling Green State University, Dr. Jan Stevenson at Michigan State University, and/or Dr. Gene Stoermer at the University of Michigan

Amphibians –

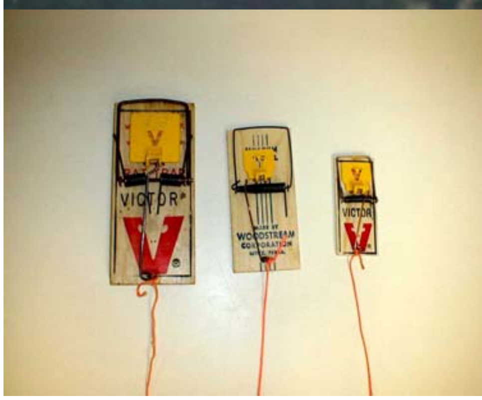
Because amphibians are extremely sensitive to weather and temperature, we assessed amphibian communities during two separate sampling periods. We conducted standardized frog-toad calling surveys[1] during the first two phenologies (early spring and late spring) between the hours of 7-10:30 PM for 10-15 minutes when water temperatures were above 50° F during the first phenology or above 60°F during the second phenology. Surveys were not conducted on evenings when strong winds or storm fronts were approaching. We used a tape recorder to record all calling and thus permitted two separate independent identifications of all calls. In addition to calling records, we added to the data records any personal observations of amphibians made during any of the daylight visits and any specimens captured during the macroinvertebrate surveys. A list of frogs and toads with their common and scientific names is provided below.

Frogs and toads expected to occur in S. E. Wisconsin wetlands (compiled by R. Hay, WDNR).

Order: Anura
- Family: Bufonidae (Toads)
  - Eastern American Toad – *Bufo americanus americanus*
- Family: Hylidae (Tree frogs)
  - Blanchard's Cricket Frog – *Acris crepitans blanchardi*
  - Chorus Frog – *Pseudoacris triseriata triseriata*
  - Spring Peeper – *Hyla crucifer crucifer*
  - Cope's Grey Treefrog – *Hyla chrysoselis*
  - Eastern Gray Treefrog – *Hyla versicolor*
- Family: Ranidae (True Frogs)
  - Bullfrog – *Rana catesbeiana*
  - Green Frog – *Rana clamitans melanota*
  - Pickerel Frog – *Rana palustris*
  - Leopard Frog – *Rana pipiens*
  - Mink Frog – *Rana septentrionalis*
  - Wood Frog – *Rana sylvatica*

[1] Wisconsin Department of Natural Resources. No Date. Wisconsin Frog and Toad Survey – Instructions, Bureau of Endangered Resources, Box 7921, Madison, WI 53707. 4 pp.

Small Mammals –



We assessed small mammal communities by trapping during a two-month period during August 14 - October 15, 2000. On each wetland we set 33 baited traps, including a mixture of 3 rat, 15 museum special grade, and 15 mouse traps (shown left to right in photo above). Traps were positioned along transects (using zig-zag scattered routes) in the riparian zone (variable dimensions, depending upon setting) for one night. Limiting the trapping effort to a single one-day/night period minimized disturbance by raccoons and other predators. Bait consisted of a mixture of peanut butter and rolled oats. Traps were cleaned and re-baited each morning. We conducted field replications at 10% of the wetlands to examine repeatability (7 wetlands sampled on different nights). Specimens were placed in labeled freezer bags and returned to the laboratory for identification. Identifications of rare taxa and species of special concern will be verified by local experts from the University of Wisconsin. A list of small mammals expected to occur in Wisconsin wetlands is included below.

Small mammals expected to occur in S.E. Wisconsin wetlands (compiled by R. Bautz, WDNR).

Order: Insectivora
Family: Soricidae (Shrews)
Masked Shrew – *Sorex cinereus*
Pygmy Shrew – *Microsorex hoyi*
Arctic Shrew – *Sorex arcticus*
Northern Short-tailed Shrew – *Blarina brevicauda*
Family: Talpidae (Moles)
Eastern Mole – *Scalopus aquaticus*
Star-nosed Mole – *Condylura cristata*

Order: Rodentia (Rodents)
Family: Sciuridae (Squirrels)
Eastern Chipmunk – *Tamias striatus*
Thirteen-lined Ground Squirrel – *Spermophilus tridecemlineatus*
Franklin's Ground Squirrel – *Spermophilus franklinii*
Southern flying Squirrel – *Glaucomys volans*
Family: Muridae (Mice, Voles, Lemmings)
SubFamily: Arvicolinae
Southern Red-Backed Vole – *Clethrionomys gapperi*
Meadow Vole – *Microtus pennsylvanicus*
Woodland Vole – *Microtus pinetorm*
Southern Bog Lemming – *Synaptomys cooperi*
SubFamily: Sigmodontinae
White-footed mouse -- *Peromyscus leucopus*
SubFamily: Mirinae
House mouse -- *Mus musculus*

Family: Dipodidae
SubFamily: Zapodidae (Jumping Mice)
Meadow Jumping Mice – *Zapus hudsonius*

Order: Carnivora
Family: Mustelidae (Weasels & Allies)
Least Weasel – *Mustela nivalis*
Short-Tailed Weasel – *Mustela erminea*
Long-Tailed Weasel – *Mustela frenata*

Data Analysis and Metric Development:

All data were transcribed from field or laboratory sheets into Excel spreadsheets or Access data files for security and record-keeping purposes. Data transcription errors were detected by comparing sums calculated using the computer (e.g., total macroinvertebrates) with manual sums as recorded on laboratory sheets. Discrepancies prompted a reexamination of the field or laboratory data (e.g., recalculation of laboratory sums) and, if no errors were found, the cross-examination of computer data entries for each and every individual taxon data for errors. Errors were corrected and sums were recalculated to confirm that the computer data matched lab totals. We further evaluated data for laboratory errors (e.g., failure to apply multiplier during subsampling of macroinvertebrates) and anomalies using a combination of univariate checks, dot histogram plots, and examination of outliers in bivariate plots prior to data analysis.

We used a combination of standard statistical and graphical procedures to analyze the data and chose appropriate metrics. Procedures included various univariate measures, Pearson correlation, ANOVA, similarity indices, multivariate, classification and ordination techniques (e.g., CANOCO of ter Braak 1988), which were available in various statistical software packages including SYSTAT (SPSS 1997), SigmaPlot 5.0 (SPSS 1998), and SigmaStat (SPSS 1992-97). Percentage data were transformed using the arc-sine square-root transformation, and abundance data were either log-transformed $(X + 1)$ or power-transformed as applicable to achieve equal variance and normality distribution assumptions where necessary (P-values for rejection of assumption testing was set at $p = 0.05$). Where transformations failed to normalize the data, we used non-parametric tests to analyze the data, including Spearman Rank Order correlation, Mann-Whitney rank sum test, and ANOVA on ranks (SigmaStat 1997). Unless noted otherwise, probability significance levels were set at $p < 0.05$.

Various community or species attributes (i.e., taxa or species richness, diversity, presence or absence of selected functional feeding guilds, trophic structure, percentages, importance values, etc.) were evaluated and scored as potential metrics based on their responsiveness to suspected measures of human disturbance (see following discussion). Community attributes were examined using a combination of procedures to select promising metrics for index development. Attributes that exhibited strong positive or negative correlation (not necessarily statistically significant) with selected human response variables were considered as prospective metrics. We compared the sensitivity and correspondence among the selected community metrics based on their performance (discriminatory ability) among both the a priori wetland impact classes and the human resource impact classes (discussed in next section), and developed an index of ecosystem integrity that best related to overall wetland condition.

This study focused attention on agricultural production (percent of watershed in agriculture land use) and the ameliorating influence of perimeter vegetative buffers (using average width of perimeter vegetation) and on urban-impacted wetlands (percent watershed in urban land use). In addition to examining possible linear relationships between the environmental attributes and counterpart biotic attributes, we also searched for evidence of threshold responses. The presence/absence of indicator taxa among three groups of agricultural intensity categories and two groups of buffer widths (protection about agricultural wetlands) were also examined. Canonical correspondence analysis was used to explore unimodal distributions of the selected biota along the various environmental gradients.

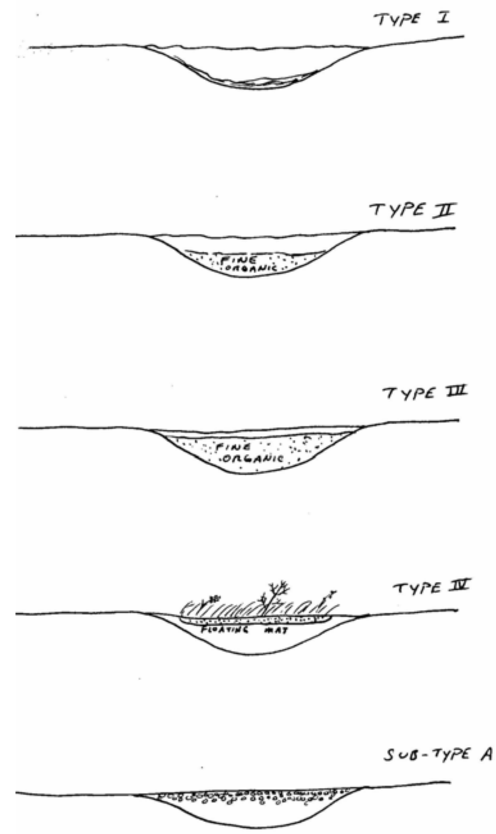
Measures of the Human Disturbance Gradient

Because different forms of human disturbance elicit different responses among the various wetland biotic communities, it was not advisable to choose one environmental measure that represented "the" single best measure of human disturbance. For example, the amphibian community may respond more directly to riparian woodland impacts (distance to nearest woodland, wooded patch size, habitat corridor dimensions, etc.) than to nutrient or pesticide loads to the wetland proper. Conversely, zooplankton and diatom communities may be more responsive to percent row crops in the watershed (a surrogate measure for pesticide and silt loading that the wetland may receive). Consequently, we evaluated several possible surrogates of human disturbance that were suspected, based on the scientific literature and research on life histories of the various component communities, to most likely influence the respective biological communities. The multitude of possible interactions (i.e., masking influences or synergistic) is enormous. Therefore, we tested a variety of attributes (and combinations of attributes) that might serve as quantitative measures of human disturbance. This included nutrients (total phosphorus and total nitrogen), chlorides, pH, and a combination of nutrients and chlorides.

Conductivity was also considered as a possible indicator of human disturbance. However, conductivity varies naturally across a broad range of alkalinity with which it is strongly co-correlated (i.e., positive association). Therefore, high conductivity values do not necessarily indicate a high degree of human impact. However, under natural situations (i.e., relatively undisturbed), conductivity and alkalinity concentrations generally are strongly correlated with one other in a 2:1 relationship across a range of values. Consequently, substantial deviations from this relationship, as indicated by comparing the magnitude of difference between measured conductivity and "predicted" conductivity based on a wetland's alkalinity value, may also represent an additional surrogate measure of human disturbance.

Water turbidity and water color were unreliable measures of disturbance in this data set. Low values may not accurately reflect inorganic or organic inputs to the respective wetlands as the result of the filtering effects of dense stands of emergent and submersed plants in some basins or the seasonal capture and release in others. A true measure of the silt load to a wetland would be a good human disturbance metric, but without more elaborate measuring devices, we can only assume the silt loading would be proportional to the amount of agricultural land use or urban construction and development in the watershed. Furthermore, any use of current land use information would miss any historical input if land use changed substantially. For example, a number of the reference prairie wetlands represented restoration sites that had previously been impacted and were now in a recovery phase. An examination of the response that sediment type (e.g., mud or peat) had on the various biological metrics failed to identify any clear relationship with the field observations of sediment type and degree of suspected human disturbance. That is to say that the physical sediment characteristics of the wetlands did not correspond with our apriori classification of wetlands nor did sediment characteristics help explain outliers among the data.

In our earlier investigations of relatively undisturbed wetlands (Lillie 2000), and also in the present study, we noted several outliers among the reference wetlands where the biological health or condition of a wetland appeared to be impaired relative to their counterparts. This was particularly true when using the macroinvertebrate-based indices. Macroinvertebrate biological index values for some reference wetlands were lower (i.e., indicating poorer condition) than values for many impacted wetlands. We speculated that either these outlier wetlands had experienced some form of unreported or unobserved (to us) human disturbance (e.g., aerial applied insecticide spraying, bait minnow stocking, or chemical treatment) or that they were ***naturally*** influenced by some environmental attribute that we had not measured or otherwise accounted for. Because macroinvertebrate taxa richness and abundance seemed to be clearly impacted (i.e., reduced) in these wetlands, we suspected that naturally lowered Dissolved Oxygen (D.O.) concentrations might be responsible (e.g., Nelson et al. 2000). Because low D.O. concentrations might occur naturally in our reference wetlands, we examined the structure of each wetland in regards to its evolutionary stage and degree of organic buildup. Based on gross observations of sediment structure (organic sediment depth and texture) and the generalized amount of open water relative to vegetative cover, we categorized each wetland into one of four wetland **stage** types (see illustration). Type I wetlands had no, or only a minimum, accumulation of organic material; the buildup present was mostly oxidized; adequate mixing and aeration are assumed, and D.O. was assumed to be adequate to support most aquatic invertebrates. Type II wetlands had a moderate accumulation of fine organic matter and was assumed to exhibit episodic periods of low D.O. Type III wetlands contained deep accumulations of fine organic material and, except perhaps for the near surface layer, was suspected to frequently experience prolonged periods of depressed D.O. Type IV wetlands had a dense floating mat of sedges with a moat about the perimeter. A subclass (Type A) was attached to each class based on the presence of a dense layer of floating leafed cover (generally based on duckweeds or watermeal). The dense layer of duckweeds or watermeal was suspected to limit reaeration and contribute to low D.O. These attributes were believed to influence the degree of oxidation and aeration occurring within each wetland, which directly or indirectly controled the D.O. levels in the wetland that are critically important for the associated macroinvertebrates and other fauna. In our subsequent examinations into the responses of individual metrics, we evaluated how wetland stage influenced metric performance.



<u>Rationale used in Metric Development</u>:

The original design of this study included the testing and further evaluation of the macroinvertebrate and plant biotic indices developed during our preliminary investigations[2]. The

[2] Lillie, R. A. 2000. Development of a biological index and classification system for Wisconsin wetlands using macroinvertebrates and plants. Final Report to USEPA Region V, Wetland Grant #CD985491-01. Wisconsin Department of Natural Resources, Bureau of Integrated Science Services, 1350 Femrite Drive, Monona, WI 53716. 50 pp + figures and appendices.

original indices and selection of metrics were based on a mixture of depressional wetlands representing a broad range of water duration, ranging from very short (episodic) to very long (persistent or permanent). Of the 104 wetlands in the original data set, only 36 were long duration basins (i.e., ≥ 7.5 month hydroperiod). Consequently, metric selection for the preliminary macroinvertebrate biotic index was highly influenced by the fact that a majority of the wetlands had short or moderate hydroperiods. Examination of the distribution of index scores showed that long duration reference wetlands generally had lower macroinvertebrate index scores (i.e., suggesting some degree of impairment) than reference wetlands with shorter water duration. This apparent bias suggested that modifications to the macroinvertebrate biotic index were warranted for long duration wetlands.

Justification for modifying the macroinvertebrate biotic index was emphasized further when index scores were calculated for the 2000 data. The majority of reference wetlands rated as 'poor' using the preliminary index, and reference wetlands did not differ from impacted wetlands (ANOVA, by type or subclass, $p>0.05$). Because all wetlands sampled during 2000 were long duration wetlands, it was necessary to modify the original macroinvertebrate index to create an index that would account for the basic differences in community structure between long and shorter duration hydroperiods.

We first reevaluated the original 1998 data using only the 36 long duration basins in an attempt to create a new macroinvertebrate biotic index that would be appropriate for assessing the condition of long duration basins only. However, only 12 of the 36 long duration wetlands in the 1988 data set represented least-disturbed reference sites. Therefore, we developed the new macroinvertebrate index using the 2000 data set and evaluated its performance using the 1998 data. A more extensive of the process is presented in the Macroinvertebrate Biotic Index section of this report.

<u>Additional Concerns</u>:

The preliminary set of indices developed for Wisconsin wetlands included two multimetric indices based on macroinvertebrates and a third multimetric index based on plant community attributes. The first macroinvertebrate-based index incorporated 15 individual metrics (derived from a total count of organisms present), while the second macroinvertebrate index included a set of ten metrics (derived from a fixed count of the first 100 organisms encountered). The 100-count method was intended to serve as a rapid field bioassessment technique. However, based on serious statistical concerns associated with fixed-count methodology (Courtemanch 1996, Barbour & Gerritsen 1996, Vinson & Hawkins 1996, Somers et al. 1998, Growns et al. 1997, Larsen & Herlithy 1998, Cao et al. 1998), we decided to discontinue investigating this procedure.

The plant biotic index has nine individual metrics (based on transect data from 18 sample quadrats). Details regarding the development of this index (including a list of attributes tested as potential metrics) are provided in an earlier report (Lillie 2000). A list of the individual metrics comprising the original macroinvertebrate index and the plant index are provided in this report.

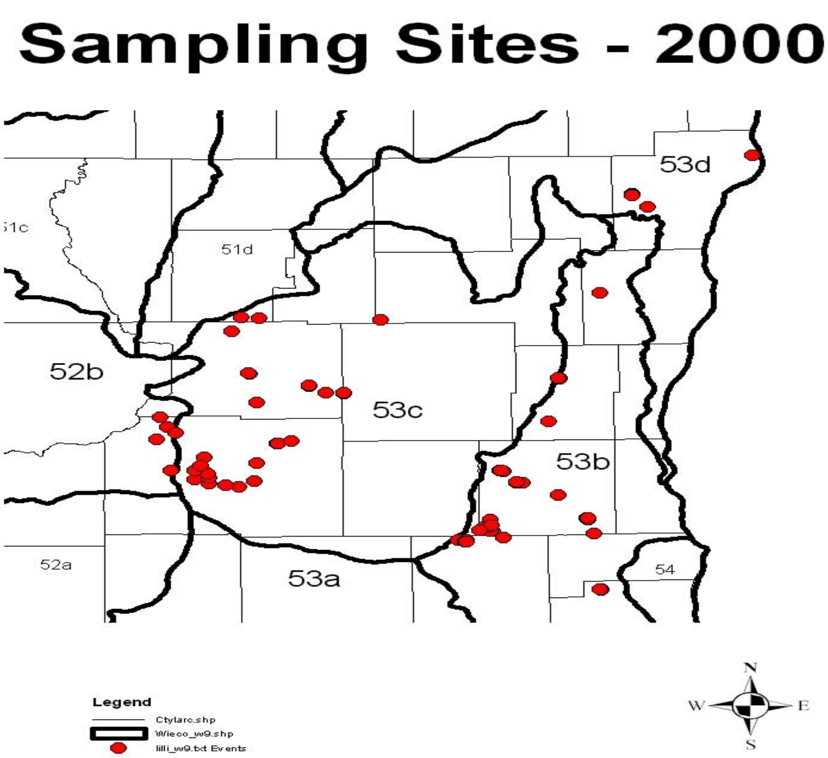
Index evaluations, refinement, calibration of scores, and development of new indices using additional biotic components generally followed procedures outlined in Gerritsen et al., 1998 (USEPA-Lake and Reservoir Bioassessment and Biocriteria Technical Guidance Document).

FINDINGS

Site Locations:

Seventy-four basins were sampled in four level IV ecoregions (per Omernik et al. 2000) in southeastern Wisconsin (Map 1, below) during 2000. The majority of wetlands were located in the Kettle Moraines (53b) and Southeastern Wisconsin Savannah and Till Plain (53c), with only six wetlands in the Lake Michigan Lacustrine Clay Plain (53d) and four wetlands in the Coulee region (52b) of the Driftless Area. A majority of the urban sites were clustered in the vicinity of the city of Madison (Dane County). GPS coordinates and town-range-section locations are provided in Appendix L – Location. Wetlands were assigned alpha-numeric codes to assist in identifying data points during graphical analysis. Basin names were assigned on the basis of their association with neighboring highways, cities, waterbodies, parks, or property owners and do not necessarily reflect wetland waterbody characteristics. For example, the French Creek basin is not a riverine wetland, but rather derives its name from being located on The French Creek State Wildlife Area).

Map 1. Location of wetlands sampled during 2000. Note: some data points overlap due to scale used. Numbers refer to Omernik et al. 2000 level IV ecoregions.



Wetland Characterization:

Impact Classification:

Wetlands were classed subjectively (i.e., a priori) into one of three disturbance classes as presented in Table 1. Wetlands believed to be subject to any form of agriculture inputs in their watersheds were classed as agriculturally-impacted (20), and wetlands situated in urban watersheds were classed as urban-impacted (18). Our earlier investigations identified a clear distinction in the biota and water chemistry between reference least-disturbed wetlands situated in either a kettle or prairie setting. Therefore, we maintained the separation of the 38 least-disturbed reference basins into kettle (19) and prairie (17) basins. Kettles represented basins located in steeply sloped morainal depressions that were generally in woodland cover. Prairie basins represented depressions in more gently sloped settings in pitted outwash plain currently in some form of grassland cover. Several prairie basins experienced some form of past agricultural production (i.e., restoration projects), while others represent a relatively natural, undisturbed condition. Appendix A provides a detailed description of each study site.

Water Duration, Hydrogeology, Depth, and Size:

All basins were of the persistent or semi-permanent and permanent class. Only two basins (Fish Lake agricultural depression and Hwy Z kettle) dried out before the end of the summer. Water depths ranged from 25cm to over 110 cm during the first sampling period, and surface water areas ranged from 0.01 to 6.8 acres. All wetlands were classed as depressional, palustrine and were of a mix of aquatic bed, emergent, and forested subclasses (classes of Cowardin et al. 1979).

Riparian Zone Characteristics:

Note: The riparian zone was defined as the area surrounding each basin from the water's edge to a distance of 30 meters. The buffer zone vegetation cover type around agriculturally impacted wetlands was neither identified nor quantified and may differ substantially from the riparian cover.

Approximately half (53%) of all studied wetlands had predominantly wooded riparian areas. All wooded kettles and a smaller percentage of each of the other wetland classes had woods as the predominant riparian cover type. Grassland was the dominant riparian cover type within the prairie reference wetlands, and grassland cover was the dominant cover type in 23% of all wetlands overall. Shrub-land (12%) and adjacent wetlands (4%) were occasionally important riparian land cover types. Agriculture (16%) and urban areas (16%) mostly were dominant only in the riparian zone surrounding agriculturally impacted and urban impacted wetlands, respectively.

Some riparian data contrast with the land use type classification based on the watershed characteristics. Agriculture land use was moderately high in the riparian area around one reference kettle (Blandings #9) and two restored prairie wetlands (Collins North and Collins South). Similarly, small areas (10-30%) of urban land use were noted on two agriculture wetlands (Mielke Road and Tompkins Road), one reference kettle (Railroad kettle), and three prairie wetlands (Laphams #s 2-4). These fore-mentioned examples are exceptions but not misclassifications because the predominant land use in their watersheds (extending beyond the riparian zone) were of the type classification.

The percent agriculture land cover in the riparian zone among the agriculturally impacted wetlands was significantly higher in heavily impacted (i.e., high agriculture intensity in watershed) wetlands than in the lightly impacted (i.e., low intensity agriculture intensity) wetlands (ANOVA, $p < 0.05$).

Vegetative Cover within the Basin:

The dominance structure of the major wetland plant cover types differed according to the watershed land use characteristics. Impacted wetlands had significantly higher percentages of open-water cover (i.e., lower percentage of plant cover) than reference wetlands (ANOVAs $p < 0.05$). Reference kettles had significantly higher emergent cover than both impacted wetland classes and prairie reference wetlands. Reference wetlands had higher floating-leafed cover than impacted wetlands. Among the agriculturally impacted wetlands, lightly impacted wetlands had significantly lower percentages of open-water area (i.e., greater total vegetative cover) than the heavily impacted wetlands.

Bottom Substrate Characterization:

Most wetland basins had predominantly mud bottom substrates (76%), while only 18% had predominantly organic substrates. Reference kettles, collectively, had the lowest percentage of mud-substrate bottoms (63%) and highest occurrence of predominantly organic substrates (26%) (Table G1).

Table G1. Frequency of occurrence of predominant bottom substrate by wetland disturbance.

Substrate	Wetland Type			
	Kettles	Prairies	Urban	Agriculture
Mud	63%	76%	78%	85%
Organic	26%	23%	17%	5%
Other	10%	1%	5%	10%

Wetland Stage:

The accumulation of organic matter and the development of dense mats of floating-leafed duckweeds, filamentous algae, or floating sedge mats may have influenced habitat conditions supporting some assemblages and their respective biotic index scores. Five of the six basins with floating sedge mats (Type IV) were reference kettles (Table G2). Over half of all basins (55%) were of the type II (moderate organic buildup) or type III (heavy organic accumulation) stage category, which were suspected to experience some form of periodic or episodic D.O. depletion. Only two (10%) of the least-disturbed kettle basins were classed as type I (minimal organic accumulations), and one of these two basins experienced extensive duckweed blooms (subclass 'a'). In comparison, 30-59% of the other three wetland classes (i.e., prairies, urban and agriculturally impacted) were of the type I stage (i.e., minimal organic accumulations), suggesting that lowered organic buildup relative to higher inorganic inputs was the mode in these basins.

Table G2. Distribution of basins according to disturbance class and wetland stage (see Fig 3 for explanation of stage typology).

Disturbance Class	Wetland Stage					
	I	Ia	II	IIa	III	IV
Reference Kettles	1	1	7	4	1	5
Reference Prairies	9	1	5	2	0	0
Urban impacts	9	0	7	0	2	0
Agricultural impacts	5	1	10	3	0	1

Presence of Vertebrate Predators:

The presence of vertebrate predators is known to influence invertebrate community composition and abundance (e.g., Brooks & Dodson 1965, Hanson & Butler 1994, Lodge et al. 1987, Schneider & Frost 1996, Corti et al. 1997, Batzer et al. 2000), and consequently the presence of predators likely would affect biotic index scores. Wetlands were coded as to the type of predators present as follows: C = crayfish, F =complex fish community with planktivores present, M = minnows only, and S = salamanders and newts. The presence of frogs (and polywogs) was not indicated. Twenty-seven basins contained some form of vertebrate predator (Table G3). These records included captures (i.e., incidental collections during net-sweeps) and visual observations only. Because we did not make a concentrated effort in documenting the presence of predators (i.e., no traps or seining used) we undoubtedly overlooked the presence of predators in several of the basins.

Table G3. Occurrence of vertebrate predators in wetlands.

Wetland Class	Fish	Minnows	Crayfish	Salamanders	None
Reference Kettles	1	0	0	3	15
Reference Prairies	1	2	2	0	12
Urban Impacts	6	2	2	0	8
Agriculture Impacts	5	1	0	2	12

Wetland Water Chemistry:

The studied wetlands exhibited a wide range in water chemistry (Table G4). Raw data measurements are provided in Appendix C. Water chemistry varied distinctly among the wetland disturbance classes (Table G4).

The rationale used in separating least-disturbed prairie wetlands from kettle wetlands is illustrated by the differences in basic water chemistry between the two classes. Prairie basins generally had significantly higher calcium ($p = 0.023$; Kruskal-Wallis One Way ANOVA of medians on ranks), alkalinity ($p = 0.013$), and conductivity ($p < 0.001$) than counterpart kettles (Fig. 4). Prairie wetlands also had lower nitrate-nitrite concentrations ($p = 0.011$) and total nitrogen concentrations ($p = 0.016$) than agriculturally impacted wetlands (Fig 5).

Combined nutrients (total nitrogen and total phosphorus) generally were highest in agriculturally impacted wetlands (Fig 6). The higher conductivity measured in urban wetlands ($p < 0.001$) is believed to be associated with the very high chlorides ($p < 0.001$; Fig.7) entering these basins in the form of road-salt runoff from the highly impervious watersheds. Urban wetlands also tended to have generally lower color levels (n.s. however) than the other wetland classes (Figure 6). Acidity, as indicated by pH values, was significantly lower in reference kettles than in urban or agriculturally impacted wetlands ($p = 0.011$; Fig 6); however, the mean for the group was roughly circumneutral and only two kettles had pH values below 6.5 units. Agriculturally impacted wetlands had the highest concentrations of TKN and total phosphorus among the groups, but the medians were not significantly different. Dissolved silica was marginally different among the groups ($p = 0.055$), and was highest in Prairie reference systems.

Table G4. Summary of basic water chemistry values for wetland disturbance classes. Data excludes replicates. Data represent mean ± 1 S.E.

Attribute (measurement)	Kettles N=19	Prairies N=17	Urban N=18	Agriculture N=20
Calcium (mg L^{-1})	19 ± 4	32 ± 4	22 ± 4	26 ± 5
Chloride (mg L^{-1})	4 ± 1	4 ± 2	97 ± 29	10 ± 3
Color (Pt-C units)	80 ± 6	80 ± 6	70 ± 8	85 ± 10
Conductivity (umhos cm^{-1})	158 ± 31	281 ± 27	476 ± 88	261 ± 47
.pH (Units)	7.24 ± 0.21	7.82 ± 0.15	8.12 ± 0.26	7.98 ± 0.21
Alkalinity (mg L^{-1})	77 ± 16	135 ± 14	88 ± 15	104 ± 19
NO2 & NO3 (mg L^{-1})	0.013 ± 0.003	0.008 ± 0.002	0.376 ± 0.361	0.769 ± 0.379
TKN (mg L^{-1})	2.361 ± 0.276	2.203 ± 0.439	2.086 ± 0.356	3.254 ± 0.519
TP (mg L^{-1})	0.231 ± 0.047	0.280 ± 0.085	0.399 ± 0.188	0.859 ± 0.265
Silica (mg L^{-1})	1.93 ± 0.48	5.68 ± 1.21	3.13 ± 1.08	2.91 ± 0.66
TN (mg L^{-1})	2.373 ± 0.276	2.211 ± 0.44	2.461 ± 0.457	4.023 ± 0.532
Combined TN+TP (mg L^{-1})	2.604 ± 0.302	2.490 ± 0.522	2.861 ± 0.586	4.882 ± 0.692

Figure 4. Box plots of alkalinity, conductivity, calcium, and dissolved silica in 74 Wisconsin wetlands sorted by a priori impact classifications. Codes are A = agriculture, K = reference kettles, P = reference prairie wetlands, and U = urban sites.

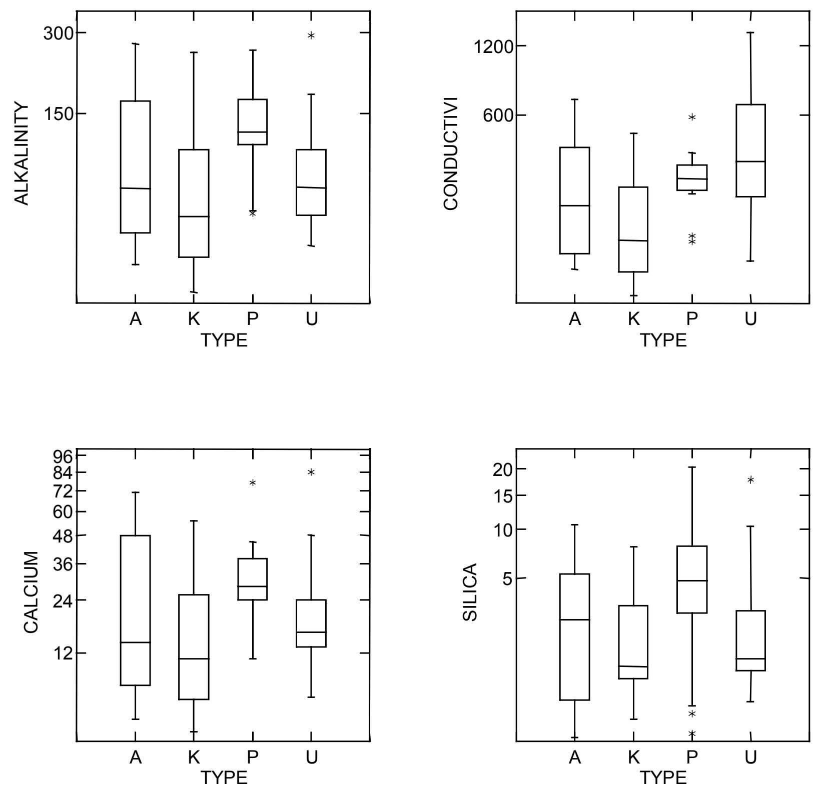


Figure 5. Box plots of nitrite-nitrate-nitrogen (NO_2 & NO_3), total Kjeldahl nitrogen (TKN), total nitrogen (TN), and total phosphorus (TP) in 74 Wisconsin wetlands sorted by a priori impact classifications. Codes are A = agriculture, K = reference kettles, P = reference prairie wetlands, and U = urban sites.

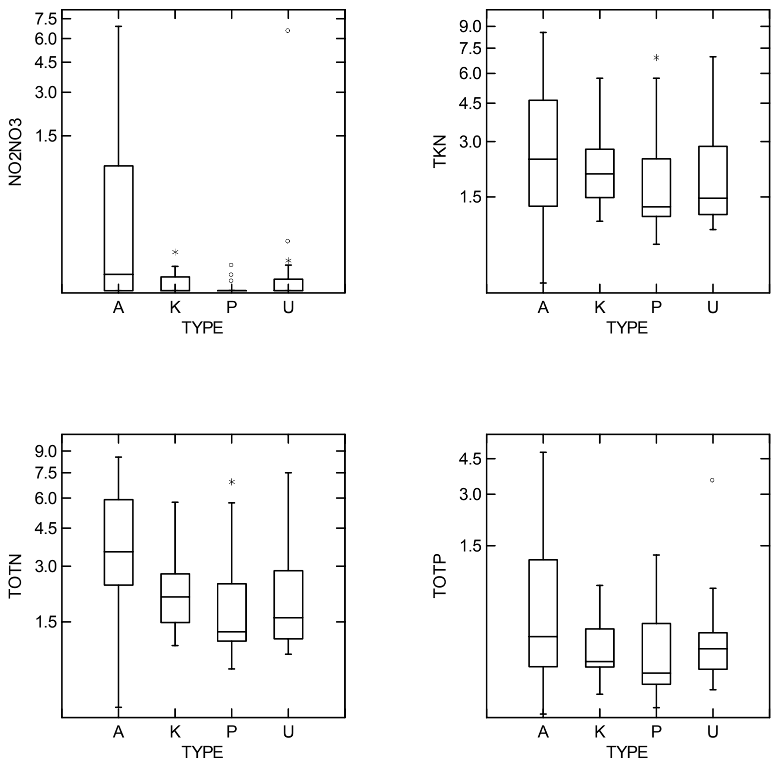


Figure 6. Box plots of color, pH, sum of total nitrogen and total phosphorus (NANDP), and deviation of measured conductivity from predicted conductivity based on alkalinity (COND_ALK) in 74 Wisconsin wetlands sorted by a priori impact classifications. Codes are A = agriculture, K = reference kettles, P = reference prairie wetlands, and U = urban sites.

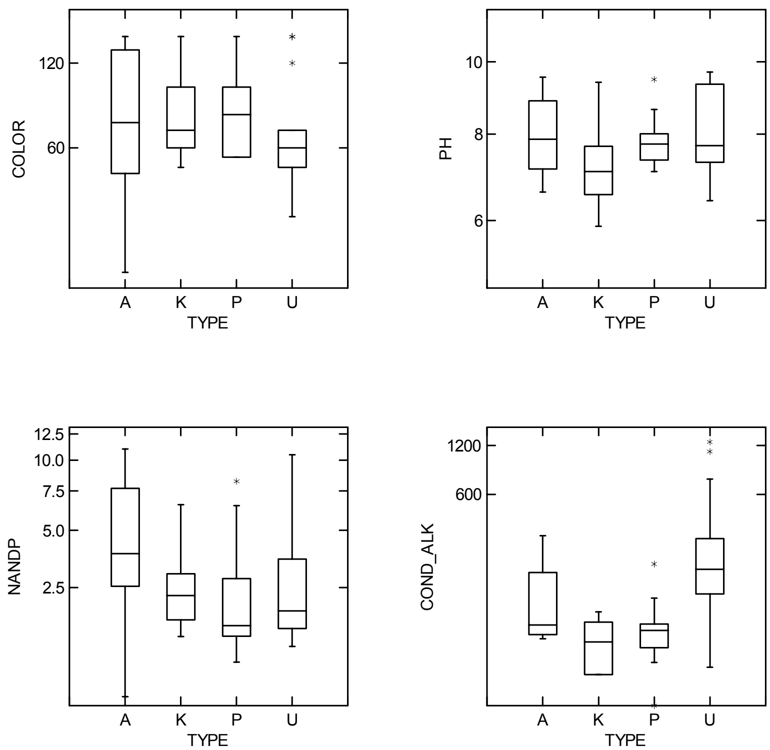
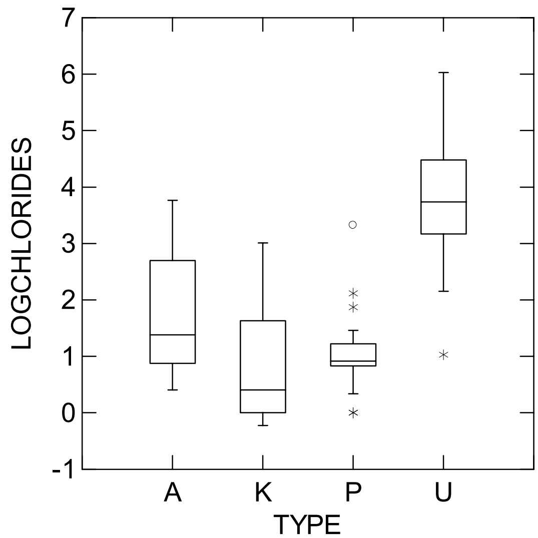


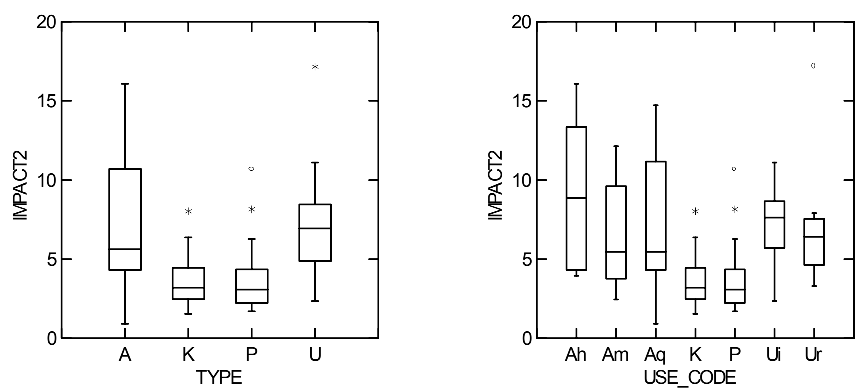
Figure 7. Box plots of chloride concentrations in 74 Wisconsin wetlands sorted by a priori impact classifications. Codes are A = agriculture, K = reference kettles, P = reference prairie wetlands, and U = urban sites.



The Human Resource Disturbance Gradient

The composite chemical index (labeled 'Impact2' in some figures below) that serves as a surrogate measure to quantify the human resource gradient (represents the combined measures of total nitrogen, twice total phosphorus, and the log-transformation of chlorides) provided excellent separation among the impacted and reference wetlands. Both agriculturally impacted and urban impacted wetlands had significantly higher impact values than reference prairies and kettle wetlands ($p < 0.001$; K-W One-way ANOVA of medians on ranks; Fig HD1). No significant differences in the composite impact measure were found between either set of impacted (A vs. U) or reference wetlands (K vs. P). However, the two groups of impacted wetlands did differ as to the major type of impact (i.e., nutrients in Agriculture sites and chlorides in Urban sites).

Figure HD1. Box plots of composite variable (impact2) representing the human resource gradient by wetland type class and use codes.



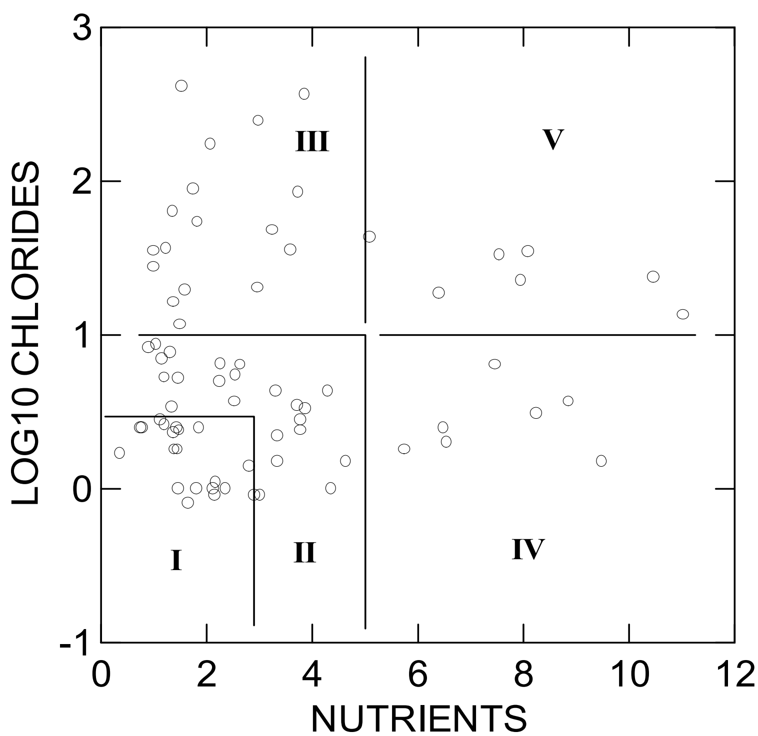
A bi-plot of the component attributes nutrients and chlorides (log-transformed) demonstrated that some wetlands with low to moderate nutrient concentrations had high chloride concentrations, and conversely some wetlands with high nutrient concentrations had low chloride concentrations. Consequently, we developed a categorical system to classify impact based on the combination of nutrient and chloride as shown in Table HD1 and Fig HD2. Class I wetlands have low nutrients and low chlorides. Class II wetlands have low to moderate nutrients and low to moderate chlorides. Class III wetlands have low to moderate nutrients and high chlorides. Class IV wetlands have high nutrients and low to moderate chlorides. Class V wetlands have both high nutrients and high chlorides.

Table HD1. Distribution of wetlands by impact type and impact class. Numbers in parentheses indicate the number of wetlands in each class for which replicates for water chemistry were taken.

Wetland Type	Impact Classification *				
	I	II	III	IV	V
Nutrients	low	low/moderate	low/moderate	high	high
Chlorides	low	low/moderate	high	low	high
Ag-Impacted	2	8 (1)	1	4	5
Kettles	8 (1)	9 (2)	1	1	0
Prairies	9 (1)	5	1	2	0
Urban-Impacted	1	1	14	0	2 (2)

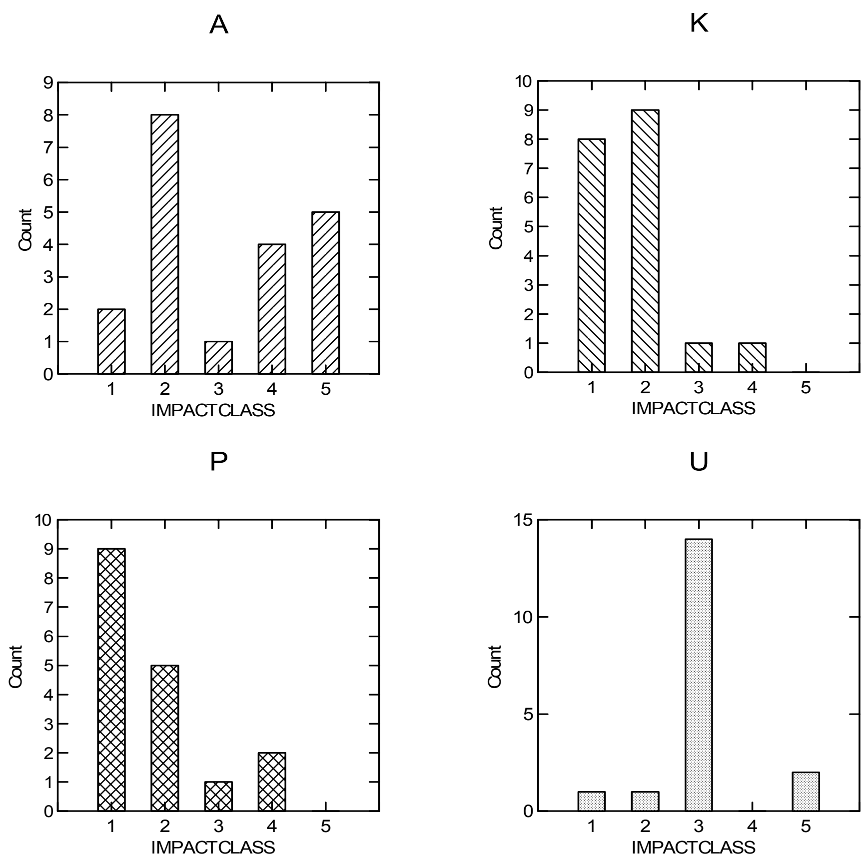
* Chloride cutoffs at 3 Mg/L and 10 Mg/L; Nutrient cutoffs at 3 Mg/L and 5 Mg/L.

Figure HD2. Impact Classification of wetlands based on chloride and total nutrient concentrations.



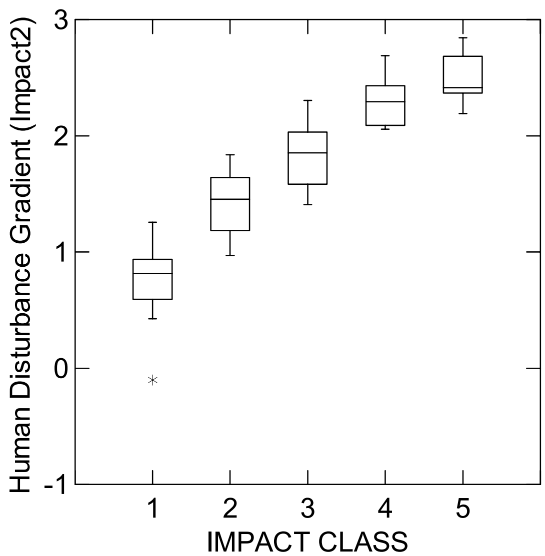
A majority of kettles (90%) and prairies (82%) rated as Class I or II, while only half of the agriculturally impacted and only 11% of the urban impacted wetlands rated so well (Fig. HD3. Roughly half (45%) of the agriculturally impacted wetlands rated as either Class IV or V, while 78% of the urban impacted wetlands rated as Class III.

Figure HD3. Distribution of wetlands by impact class and a priori impact classification.



Impact values increased progressively from Class I to Class V (Fig. HD4) and were statistically different among each of the classes (ANOVA, $F_{4,69} = 74.8$, $p < 0.001$; all pairwise comparisons $p < 0.1$).

Figure HD4. Box plots of impact values by impact classification.



An undetermined number of the 14 impacted wetlands that fell into the Class I or Class II ranks most likely received other forms of pollutants (e.g., herbicide-insecticide, heavy metals, thermal, etc.) not reflected in either nutrient or chloride concentrations. Likewise, nutrient concentrations in some wetlands may have been reduced by dense stands of emergent or submersed vegetation (i.e., nutrient uptake and incorporation into vegetative tissue).

Macroinvertebrates:

NOTE: The level of taxonomy used to identify and analyze the macroinvertebrate data in this study was set intentionally at a very coarse level (mostly order and family) in order to allow the procedures to be applied by field staff with a minimum of scientific training.

General Characteristics:

Over 26,000 macroinvertebrates were collected from 75 wetlands. The tally includes specimens from seven replicates and one wetland later dropped from the sampling program. Total macroinvertebrate abundance in individual samples ranged from as few as 6 to nearly 3,500 specimens (Table M-1). Average total abundance was 325 specimens per sample, with roughly half of these being insects and the other half a mixture of mollusks (snails and clams) and various crustaceans.

Taxonomic richness ranged from 1 to 28 taxa groups (a mixture of taxonomic levels or morphs as explained in the methods), with a median of 14 taxa per sample. Taxonomic composition of the samples varied considerably among the individual wetlands. Diptera (Order of the true flies) represented the dominant insect order in 79% of the wetlands. Chironomidae (non-biting midges) was the most common dominant taxonomic group (32%), and Culicidae (mosquitoes) was a distant second (10%). Of the 45 taxonomic groups identified in the sorting process, the most commonly occurring taxa by rank were chironomids, mollusks (snails and clams combined), snails, Ceratapogonidae (biting midges), Dytiscidae (predaceous diving beetles), Haliplidae (crawling water beetles), clams, Odonates (combined dragonflies and damselflies), and Pleidae (pygmy backswimmers) (Table M-2). A list of macroinvertebrate taxa found in each wetland is provided in Appendix C.

Table M-1. Descriptive statistics for selected macroinvertebrate summary groups.

Community Attribute	Range	Median	Mean ± 1SE	C.V.%
Insect Abundance	0-840	103	164 ± 21	114%
Total Invertebrates	6-3497	168	325 ± 55	152%
Insect Richness	1-19	9	9 ± 0.4	40%
Non-insect Richness	0-11	5	5 ± 0.3	50%
Total Richness	1-28	14	14 ± 0.6	37%
Diversity	0.001-4.19	2.49	2.47 ∀ 0.10	35%

Table M-2. Frequency of occurrence of 25 most commonly occurring macroinvertebrate taxa groups. Data represent percent of 80 samples containing at least one specimen.

Taxonomic Group	Frequency of Occurrence
Diptera: Chironomidae (non-biting midges)	95%
Mollusks (snails & clams)	86%
Gastropoda (Snails only)	85%
Diptera: Ceratopogonidae (biting midges)	82%
Coleoptera: Dytsicidae (Predaceous diving beetles)	71%
Coleoptera: Haliplidae (Crawling water beetles)	63%
Odonata (Anisoptera & Zygoptera)	62%
Hemiptera: Pleidae (Pygmy backswimmers)	60%
Coleoptera: Hydrophilid (Water scavenger beetles)	59%
Hirudina (Leeches)	56%
Hemiptera: Corixidae (Water Boatmen)	56%
Annelida (Worms)	51%
Amphipoda (Scuds)	48%
Odonata: Zygoptera (Damselflies)	47%
Diptera: Stratiomyiidae (Soldier flies)	46%
Hydracarina (Water mites)	39%
Trichoptera All Caddisflies	39%
Isopoda (Sowbugs)	34%
Diptera: Culicidae (Mosquitoes)	33%
Limnephilidae (a cased caddisfly)	30%
Ephemeroptera All mayflies	26%
Caenidae (Squaregilled mayflies)	23%
Diptera: Chaoboridae (Phantom midges)	20%
Anostraca (Fairy shrimp)	16%
Coleoptera: Scirtidae (Marsh beetles)	8%

Macroinvertebrate Biotic Index Development and Refinement

The preliminary Wisconsin Wetland Macroinvertebrate Biotic Index (WWMBI) is a multimetric index incorporating 15 individual metrics (69 community attributes were tested as potential metrics), including 12 abundance metrics, two richness metrics, and one percentage metric (Table M-3). The WWMBI was developed from a set of 104 depression wetlands distributed across several ecoregions (please refer to Lillie 2000 for more details). The wetlands in the development data set represented a variety of wetland hydrogeomorphic types and a wide range in water duration (i.e., hydroperiods). The WWMBI was intended to serve as only one tool among others in rating, ranking, and comparing wetland biological condition among Wisconsin depression, palustrine, wetlands. The objectives of the current study included evaluating the performance of the WWMBI using an independently selected set of wetlands and modifying the index if it was determined that modification was deemed necessary.

Table M-3. Assignment of scores for macroinvertebrate metrics included in the **Preliminary** Wisconsin Wetland Macroinvertebrate Index (WWMBI). Details on development are provided in Lillie 2000).

Taxa Group	**Attribute**	**Limitations**	**Response**	**Scores: 0**	**1**	**3**	**5**	**Modifi-cations**
Mollusks	Abundance	None	Decrease	0	1-10	11-99	>99	-
Annelids	Abundance	none	Decrease	-	0-10	11-25	>25	-
Fairy Shrimp	Abundance	Short-med. duration	Decrease	-	0-8	9-25	>25	8-9 months = 5
Non-insects	Richness	Useful in prairies	Decrease	0	1-2	3-5	>5	-
Damselflies	Abundance	Useful in kettles	Decrease	0	1-2	3-15	>15	-
Pigmy backswimmers	Abundance	Long duration only	Increase	0	1-2 and >100	3-5 and 11-99	6-10	< 7 months = 5
Water boatmen	Abundance	none	Decrease	0	1-4	5-10	>10	-
Limnephelids	Abundance	Med. water duration?	Decrease	0	1-10	11-50	>50	-
Caddsiflies	Percent	Redundant?	Decrease	0	<8%	8-15%	>15%	> 7 months?
Caddisflies	Abundance	May need duration adjustment	Decrease	0	1-10	11-60	>60	? < 4 months
Phantom midges	Abundance	Longer duration only	Decrease	0	1-8	9-25	>25	< 4 months = 5
Mosquitoes	Abundance	Short-med. duration?	Decrease ?	0	1-10	11-99	>100	?
Soldier Flies	Abundance	Long-med. duration?	Increase	-	<25	8-24	<7	< 4 months not used
Total Invertebrates	Abundance	none	Decrease	<150	150-500	500-1500	>1500	
Total Taxa	Richness	Base adjustment for kettles vs	Decrease	<5	6-11	12-19	>19	

		prairies?						

The following paragraphs present a chronology of the steps taken during the evolution of the WWMBI into a functional index that is restricted for use on longer duration depression wetlands. The detailed explanation captures the thought and effort that went into this iterative process and illustrates the complexity of issues involved. Biotic index development is not a simple, straightforward procedure. Rather it requires careful examination of the data and, frequently, de-emphasis of statistics in favor of rationalization of the biological meaning of the various contributing metrics.

New Macroinvertebrate Biotic Index Development:

During the preliminary evaluation of the WWMBI using the initial developmental data (i.e., 104 wetlands sampled during 1998), it was discovered that the WWMBI performed poorly in discriminating among impacted and least-disturbed wetlands among long duration (hydroperiods ∃ 7.5 months) wetlands (Lillie 2000). Least disturbed, long duration, depression wetlands tended to score lower (representing a worse condition) than counterpart least disturbed wetlands of shorter duration. This inherent bias in the WWMBI arose from the fact that the WWMBI incorporated some abundance-based metrics for taxa that generally were restricted to shorter hydroperiods (e.g., fairy shrimp and mosquitoes). The absence (or low abundance) of these taxa in the longer duration wetlands undoubtedly led to a lower score than would be expected for a relatively un-impacted site. Consequently, we began attempts to develop an index more suitable for measuring the condition of long duration wetlands by reanalyzing the 1998 data including only those basins with long duration (N=36). We followed the same standard procedures in evaluating the response of measured biological attributes to suspected impacts as described in the earlier investigations (Lillie 2000). Those attributes that illustrated some degree of separation (based on graphic analysis of box plots by a priori disturbance classes) were selected as potential metrics. Each of the selected potential metrics was further evaluated by measuring its response to the various environmental variables using the year 2000 data set. Those potential metrics that exhibited inconsistent or only very weak responses to chloride or nutrient concentrations were dropped from further consideration. In this process, we narrowed the number of metrics used in the multimetric index from the original 15 to 11. We dropped abundance-based metrics using fairy shrimp (usually limited to shorter duration basins), pygmy backswimmers (a decision later revisted), and limnephilid caddisflies, and the single percentage metric in the original index, percent caddisflies. We assigned scores of 5, 3, 1, and 0 to each of the remaining 11 metrics using the trisection technique after transforming the metric values to achieve a fairly normal distribution. Unfortunately, because there were so few least disturbed, long duration, reference basins (N=12) in the original data set (year 1998), the newly developed scoring system when applied to the new data (year 2000) proved quite inadequate, with most reference basins receiving relatively poor ratings. Therefore, we adjusted the scoring system using a combination of the original data scores and a trisection of the new data.

During the later phase (i.e., examination of the new data abundances and assignment of new scores), we discovered that additional modifications to the index were necessary to achieve maximum discriminatory power between impacted and least disturbed reference basins. We dropped the worm (annelids), damselfly, and non-insect richness (later reconsidered), total taxa richness, and total invertebrate metrics, and added insect taxa richness and a diversity measure (Margalef's index – Magurran 1988), which essentially substituted for the total taxa richness and total invertebrate abundance metrics which were being dropped.

While testing redundancy among the remaining metrics, we discovered that, in most cases, the chaoborids (phantom midges) and culicids (mosquitoes) were mutually exclusive. This may have been related to indirect effects of predator-prey relationships occurring within the wetlands. The net effect of the negative relationship among the two taxa was that their biotic index scores canceled one another out, resulting in an averaging of their numbers and masking the significance of the their contribution to the community structure. To combat this effect, we combined the two groups into a single metric termed the Phantoms-Mosquitoes metric. This metric appeared to warrant additional scoring adjustments in cases where fish predation was sufficiently strong enough to reduce the numbers of phantom midge or mosquito larvae. Eventually, after many rounds of testing and examining the data more carefully, we found that this metric tended to mask the scores of other metrics, so eventually we discontinued applying the Phantom-Mosquitoes metric.

The richness metrics proved to be somewhat troublesome also. We vacillated between including the total taxa richness metric alone or including only one or both of the component richness metrics (non-insects and insects). A careful reevaluation of the three taxa richness metrics (i.e., non-insect, insect, and total taxa richness) revealed that although insect richness and non-insect richness were co-correlated (and therefore potentially redundant) the non-insect richness metric tended to perform better in prairie wetlands and the insect richness metric performed better in kettles. The total richness metric generally had lower discriminatory power than the component metrics. Therefore, we have included both non-insect richness and insect richness metrics in the final index.

In a final reexamination of all other potential metrics, we discovered that pigmy backswimmer abundance was a moderately useful metric (i.e., including the metric enhanced the discriminatory power of the overall index), and was consequently restored as the eighth and final metric in the new index. The pigmy backswimmer metric seemed to respond (negatively) to the amount of unaccounted or unexplained excessive conductivity attribute.

Response of Individual Metrics to Environmental Attributes and the Human Disturbance Gradient

Associations between the eight component metrics and environmental variables are provided in Table M4 below. Mollusks, water boatmen, non-insect richness (driven primarily by the mollusks), and diversity exhibited strong relationships with several environmental variables. The abundance of pigmy backswimmers appeared to be inversely related to the excessive conductivity measure. Caddisflies appeared to respond favorably to calcium and negatively to basin size. Soldier flies responded negatively to pH. Insect richness did not demonstrate a statistically significant relation with any individual environmental variable (particularly with nutrients or chlorides). The weakness or lack of significant findings between many of the associations was, in part, due to the inclusion of many zeros in the data. Multiple factors, including presence of predators and vegetative differences, contributed to the masking of direct cause-effect associations.

Table M4. Significance of non-parametric Spearman correlation coefficients among biological index metrics (all log-N transformed) and environmental attributes (untransformed unless otherwise noted). Significance level for probability values are (++) or (- -) = p < 0.05 and (+) or (-) = p < 0.10. Direction of relation given as (-) negative and (+) positive.

Attribute	Mollusks	Pigmy back - swimmers	Water Boatmen	Caddisflies	Soldier Flies	Non-Insect Ricness	Insect Richness	Diversity
Size	(- -)	n.s.	n.s.	(- -)	(- -)	(- -)	n.s.	n.s.
Depth	n.s.	n.s.	n.s.	n.s.	(- -)	n.s.	n.s.	n.s.
Calcium	(+ +)	n.s.	n.s.	(+ +)	n.s.	(+ +)	n.s.	n.s.
Chloride	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Color	(-)	n.s.	n.s.	n.s.	(+ +)	n.s.	n.s.	n.s.
Conductivity	(+ +)	n.s.	n.s.	n.s.	n.s.	(+)	n.s.	n.s.
Excessive Conductivity*	n.s.	(- -)	(+ +)	n.s.	n.s.	n.s.	n.s.	n.s.
PH	n.s.	n.s.	(+ +)	n.s.	(- -)	n.s.	n.s.	n.s.
Alkalinity	(+ +)	n.s.	n.s.	(+)	n.s.	(+ +)	n.s.	n.s.
Total Kjeldahl Nitrogen	(- -)	n.s.?	n.s.	n.s.	n.s.	(- -)	n.s.	(-)
Total Phosphorus	(- -)	n.s.	n.s.	n.s.	n.s.	(- -)	n.s.	n.s.
Dissolved Silica	(- -)	(- -)	n.s.	n.s.	n.s.	(+ +)	n.s.	n.s.
Total Nitrogen	n.s.	n.s.	(+ +)	n.s.	(-)	(- -)	n.s.	(- -)
Total Nutrients (N & P)	(- -)	n.s.	(+ +)	n.s.	(-)	(- -)	n.s.	(- -)
TKN & P	(- -)	n.s.	n.s.	n.s.	n.s.	(- -)	n.s.	n.s.
Log-N Chlorides	n.s.	n.s.	(+ +)	n.s.	n.s.	n.s.	n.s.	n.s.

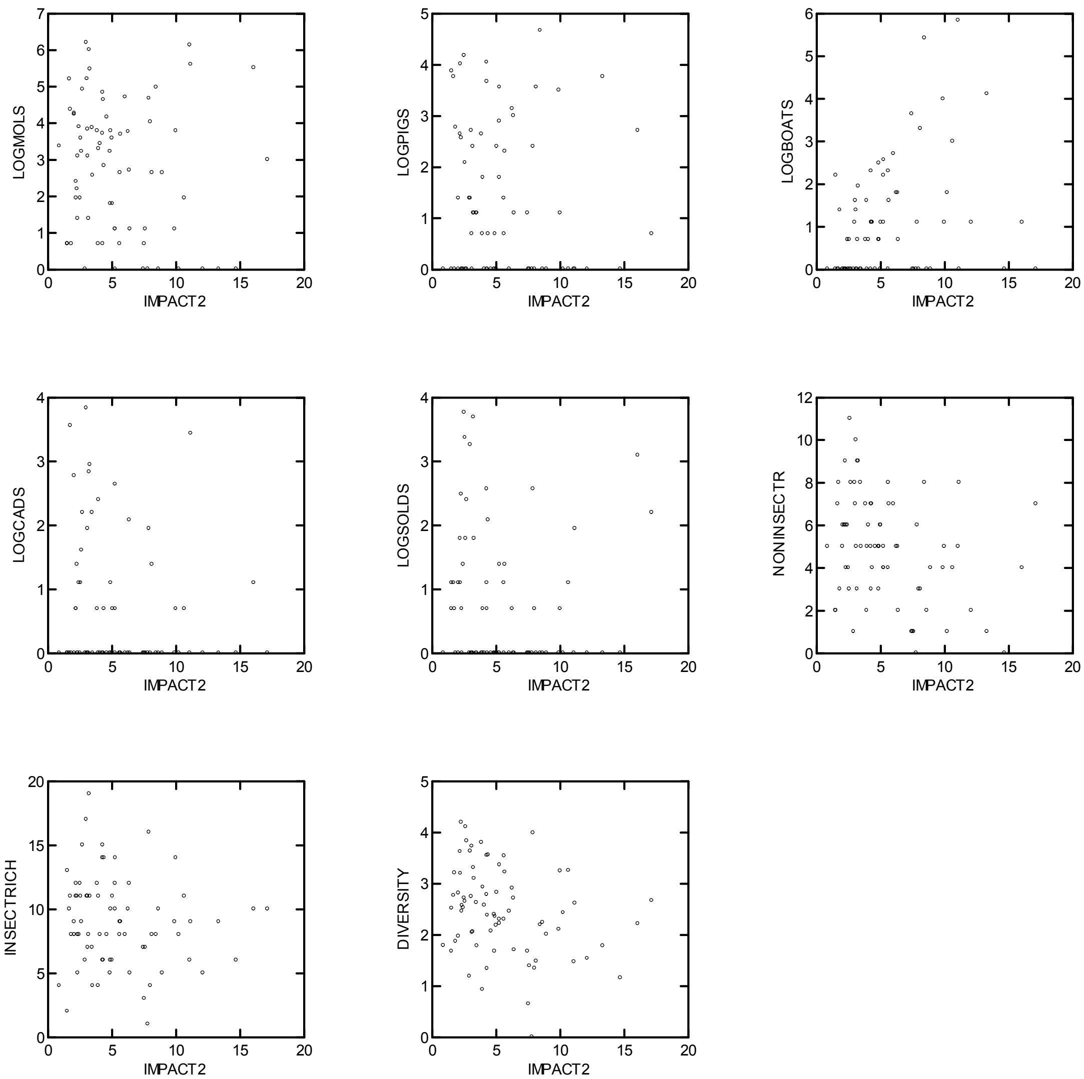
- Amount of conductivity concentration not accounted for by chlorides present as determined by the formulae: (Conductivity – (2 * (Alkalinity)) – Chlorides) = Excessive conductivity. The chemical imbalance may indicate an additional form of unaccounted human disturbance?

The associations between individual metrics and the human resource gradient are provided in Table M5 and further illustrated in Figure M1a-h. The diversity, non-insect richness, and soldier fly metrics were strongly negatively correlated with the human resource measure (all $p < 0.05$), while insect richness and total caddisflies were moderately negatively correlated with the human resource gradient ($p < 0.10$). Water boatmen were strongly positively correlated. The association between mollusks and the human resource gradient was masked by calcium and alkalinity. The pigmy backswimmer metric responded to the excessive conductivity measure, but not directly to the human resource measure.

Table M5. Relation between individual metrics and composite human resource gradient (log-transformed 'Impact') based on Spearman correlation coefficients.

Metric (Transformation used)	Direction of association and Correlation Coefficient	Comments
Mollusks (Log x+1)	(weakly negative), n.s.	Association masked by calcium and alkalinity gradient
Pigmy backswimmers (Log x+1)	(weakly negative), n.s.	Responds to excessive conductivity which is not included in the composite human resource metric.
Water Boatmen (Log x+1)	(strong positive), $P < 0.001$	Note: positive response to both nutrients and chlorides.
Total Caddisflies (Log x+1)	(moderately negative), $P = 0.053$	Primarily responding to TKN-nitrogen.
Soldier Flies (Log n+1)	(strong negative), $p = 0.015$	Primarily responding to total nitrogen.
Non-Insect Richness (untran.)	(strong negative), $p = 0.003$	Strongly responding to all nutrients, including phosphorus – minimally to chlorides.
Insect Richness (untransformed)	(moderately negative), $p = 0.071$	Primarily responding to total nitrogen.
Diversity (untransformed)	(strong negative), $p = 0.002$	Responds to both nitrogen and phosphorus.

Figure M1a-h. Bi-plots of individual metrics versus the composite human resource gradient (labeled impact2).



After each modification stage in the development of the new biotic index, we measured the improvement (or decline) in the association between the biotic index and the various environmental variables serving as the surrogate human disturbance gradient (Table M6). We also examined bi-plots of the relationships to examine the shape of the relationships. In almost every instance the final generation model exhibited the strongest associations with the environmental attributes. The association between the index and total phosphorus concentration declined with modification until the last stage when the strength of the association recovered to match that using the original WWMBI. The new index is more strongly associated with nutrients than chlorides, but the association is strongest with the compound human disturbance index – incorporating both nutrients and chlorides.

Table M6. Impact of modifications to the WWMBI on the strength of associations with environmental attributes during different stages of development. Data represent significance of associations between the index scores and environmental measures as indicated by Spearman Rank Order correlation coefficients. Exact probabilities provided beneath signs. Associations between Wisconsin Wetland Plant Biotic Index (WWPBI) and environmental measures are also provided.

Index Scores versus Environmental Variable	**Original WWMBI**	**1st Stage**	**2nd Stage**	**3rd Stage**	**4th Stage**	**5th Stage**	**New WWMBI**	**Original WWPBI**
Total Phosphorus	n.s	n.s.	n.s.	n.s.	n.s.	(-) p=0.064	(-) p=0.053	(- -) p=0.000
Total Nitrogen	(-) p=0.090	(- -) p=0.004	(- -) p=0.001	(- -) p=0.004	(- -) p=0.012	(- -) p=0.003	(- -) p=0.006	(-) p=0.062
Combined Nutrients	(-) p=0.073	(- -) p=0.004	(- -) p=0.002	(- -) p=0.004	(- -) p=0.012	(- -) p=0.002	(- -) p=0.005	(-) p=0.035
Chlorides	n.s.	n.s.	n.s.	n.s.	n.s	n.s.	n.s.	(- -) p=0.001
Impact # 1 (N+P+LogCl)	n.s.	(- -) p=0.010	(- -) p=0.002	(- -) p=0.003	(- -) p=0.002	(- -) p=0.000	(- -) p=0.001	(- -) p=0.000
Impact # 2 (N + 2P+LogCl)	n.s.	(- -) p=0.010	(- -) p=0.003	(- -) p=0.003	(- -) p=0.002	(- -) p=0.000	(- -) p=0.000	(- -) p=0.000

The NEW WISCONSIN MACROINVERTEBRATE BIOTIC INDEX for Long Duration, Depression Basin Wetlands:

The numerous modifications to the original WWMBI resulted in a modified macroinvertebrate biotic index designed for longer duration depression basin wetlands (Table M7). The new index is comprised of eight component metrics, which includes five abundance based metrics, two richness metrics, and a simple diversity metric.

Table M7. The Modified WWMBI for long duration wetlands, with scores.

Taxa Group	Attribute	Adjustments	Response	Score = 0	Score = 1	Score = 3	Score = 5
Mollusks	Abundance	Low Calcium or Alkalinity*	Decrease	0	1-9	10-99	∃100
Pigmy backswimmers	Abundance	None	Decrease	0	1-3	4-11	∃12
Water Boatmen	Abundance	Low total invertebrate abundance**	Increase	∃12	5-11	1-4	0
Caddisflies	Abundance	None	Decrease	0	1-2	3-7	∃8
Soldier Flies	Abundance	None	Decrease	0	1-2	3-9	∃10
Non-Insects	Richness	None	Decrease	0	1-2	3-4	∃5
Insects	Richness	None	Decrease	< 3	3-7	8-11	∃12
All Macro-invertebrates	Diversity*	None	Decrease	< 1	1-2	2-3	∃ 3

- If either calcium < 5 or alkalinity < 25 occurs, then substitute average of other seven metrics.
- ** If boatmen abundance < 5 and total invertebrates < 100, then substitute ‘2’.
- *** Margalef’s Diversity D = (Total Taxon Richness –1)/Log_n Total Macroinvertebrate abundance.

Scoring of New Metrics

As mentioned previously, scoring of metrics was an iterative process beginning first with the tri-sectioning of the long duration wetlands in the 1998 data set. This proved of little value because there were too few reference wetlands in the 1998 set (N=12). Consequently, we established scores of 0, 1, 3, or 5 based on the distribution of data among the reference kettles and prairie wetlands in the year 2000 set (N=38 points). Bi-plots of each metric opposed to the impact gradient were power-transformed (graphically using Systat Version 8.0, 1998) as necessary to achieve as close to a normal distribution of data points as possible prior to tri-sectioning the metric scale and assigning scores. Some of the abundance-based metrics contained many zeroes, which interfered in this effort. Scores are provided in Table M7.

The mollusk metric requires an adjustment for cases where calcium or alkalinity may be limiting the ability of mollusks to colonize the basin (Lodge et al. 1987). When either calcium < 5 Mg/L or alkalinity < 25 Mg/L, the mollusk score should be substituted with a value equal to the average of the other seven metric scores for that particular wetland.

The corixid (waterboatmen) metric is the only index metric with an inverse rating scale. The abundance of waterboatmen generally increases with increased degree of impact (Fig M1c). Corixid abundance was significantly correlated (positive) with both chlorides (log-transformed) and nutrients (Table M4). Consequently, the scores assigned to corixid abundance are reversed relative to the scale of the other seven metrics. Wetlands with low numbers (or none) of corixids receive a score of either 3 or 5 (Table M7). Low (or no) numbers of corixids may also occur in impacted wetlands if the unidentified stressor (other than nutrients) has an adverse impact on corixids. To account for this possibility an adjustment in scoring is required. When total macroinvertebrate abundance is less than 100 organisms and fewer than 5 corixids are present in a sample, the corixid metric score is set at 2.

Rating System for the new Macroinvertebrate Biotic Index

The MasterBI scores were assigned qualitative ratings based on the distribution of scores among the reference kettle and prairie wetlands (N = 38; Fig. M2). The use of the impact variable for the x-axis in the figure illustrates the relation between the surrogate human resource gradient and the MBI values within the reference wetlands. Cutoff values for the ratings were chosen to represent the 10%, 25%, 50% (i.e., median), 75%, and 90% quantiles (Table M8). The impacted wetlands were subsequently rated using the same x-y plot (Fig M2).

Figure M2. Distribution of macroinvertebrate biotic index scores for 36 reference wetlands, with rating lines illustrated.

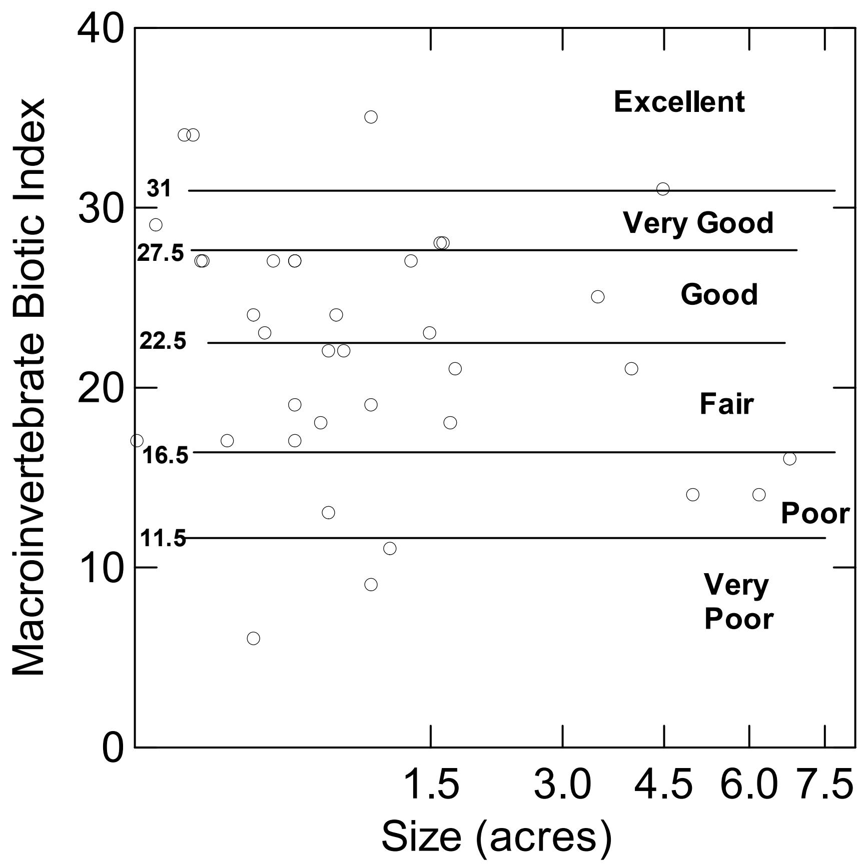


Table M8. Qualitative rating system for MBI scores based on distribution of scores within reference wetlands.

Qualitative Rating	**Range of MBI scores**
Excellent	∃ 31
Very Good	28-30
Good	23-27
Fair	17-22
Poor	12-16
Very Poor	# 11

Applying this rating system, 19% of the reference kettle wetlands and 18% of the reference prairie wetlands were rated as poor or very poor, while 50% of the ag-impacted wetlands and 55% of the urban-impacted wetlands were rated as poor or very poor (Table M9).

Table M9. Distribution of qualitative ratings among the a priori wetland impact classes.

Wetland Type	**Excellent**	**Very Good**	**Good**	**Fair**	**Poor**	**Very Poor**	**Sum**
Reference kettles	3	3	5	6	1	3	21
Reference prairies	1	2	6	5	3	0	17
Ag-impacted	0	2	2	7	3	8	22
Urban-impacted	1	2	3	3	6	5	20

When applied to the objective impact classes, the MBI classifications also match up quite well (Table M10). Approximately half (47%) of the wetlands with low-medium concentrations of chlorides and nutrients rated as good or better on the MBI scale, while only 24% of the wetlands containing substantial amounts of either chlorides or nutrients rated as well. Sixty-one percent of the impacted wetlands were rated as poor or very poor, while only 19% of the wetlands in the low-medium classes rated poor or very poor.

Table M10. Distribution of qualitative MBI ratings among objective impact classes.

Wetland Class	**Excellent**	**Very Good**	**Good**	**Fair**	**Poor**	**Very Poor**	**Sum**
Low-low	1	3	6	4	5	2	21
Med-med.	3	4	5	12	0	2	26
Chlorides	1	0	4	4	3	5	17
Nutrients	0	0	0	1	2	5	8
Both	0	2	1	0	3	2	8

Among land use classes, seven of the ten urban-industrial impacted wetlands rated as poor or very poor and four of the ten urban-residential impacted wetlands rated similarly (Table M11). The percentage of wetlands rated as poor or very poor increased along the gradient from light to high impacts.

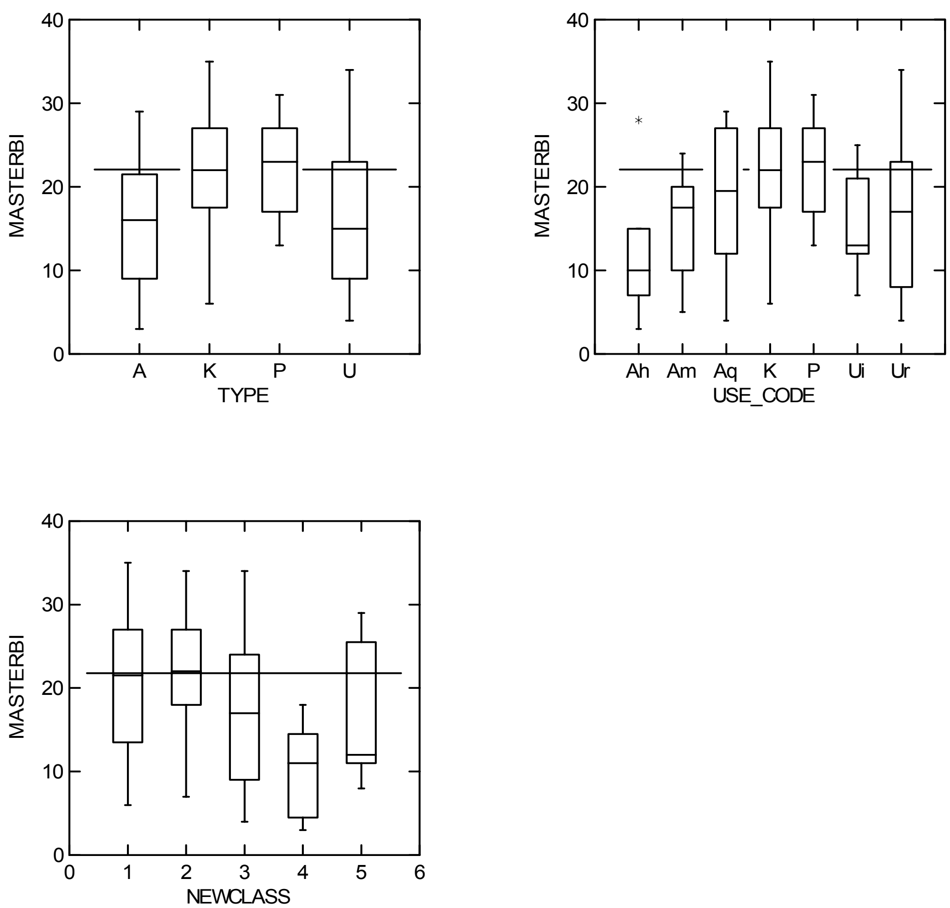
Table M11. Distribution of qualitative MBI ratings among impacted land use categories.

Land use category	**Excellent**	**Very Good**	**Good**	**Fair**	**Poor**	**Very Poor**	**Sum**
Ag-High	0	1	0	0	1	4	6
Ag-Medium	0	0	1	4	1	3	9
Ag-Light	0	1	1	3	1	1	7
Urban-industrial/commercial	0	0	2	1	5	2	10
Urban-residential	1	2	1	2	1	3	10

Performance of the Macroinvertebrate Biotic Index as modified for Long Duration Wetlands

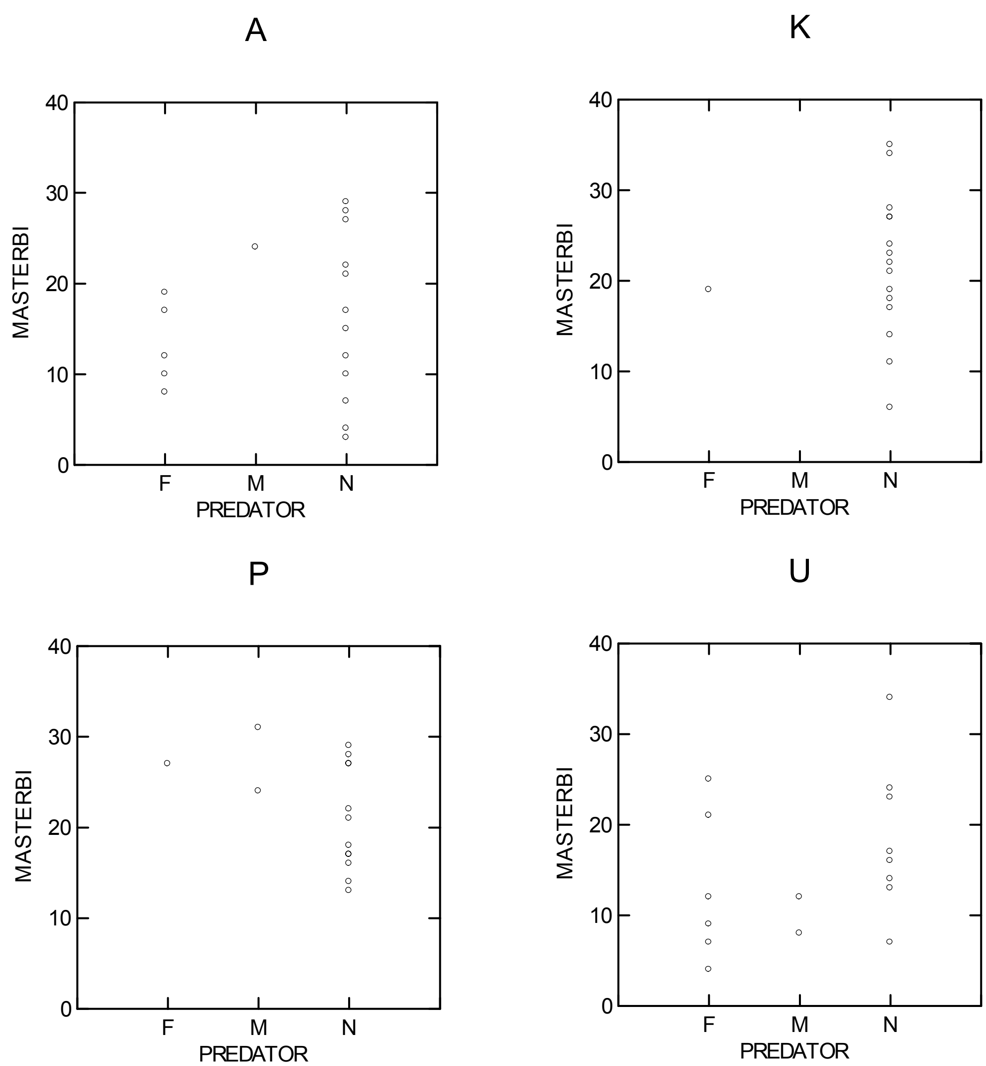
We used ANOVA (or Kruskal-Wallis AVOVA on Rank Order) to measure the ability of the MBI to distinguish differences among the various wetland classifications. MBIs differed according to wetland type (p=0.004), impact class (p<0.001), and land use categories (p=0.012). Subsequent Tukey analysis indicated the following significant differences. Among the four basic wetland types (Fig. M3a), MBI scores in reference wetlands (both kettles and prairies) were significantly higher than in agriculturally impacted wetlands (p = 0.015 and p = 0.039, respectively). MBI scores in reference kettles were marginally higher (p=0.054) than in urban impacted wetlands. The difference between MBI scores in reference prairies and urban impacted wetlands was not statistically significant at the p=0.10 level. Among the finer resolution land use groupings (Fig M3b), and despite the overall findings of significance in the model, reference kettles had marginally higher MBIs than highly ag-impacted wetlands (p=0.074). Other comparisons were not significant at the p=0.10 level. Among impact classes (Fig M3c), MBIs in both the medium-medium and low-low impact classes were significantly higher than in class IV nutrient impacted wetlands (p <0.001 and p=0.002, respectively).

Figure M3. Box plots of the macroinvertebrate biotic index (MasterBI) by wetland class, use code, and impact classification.



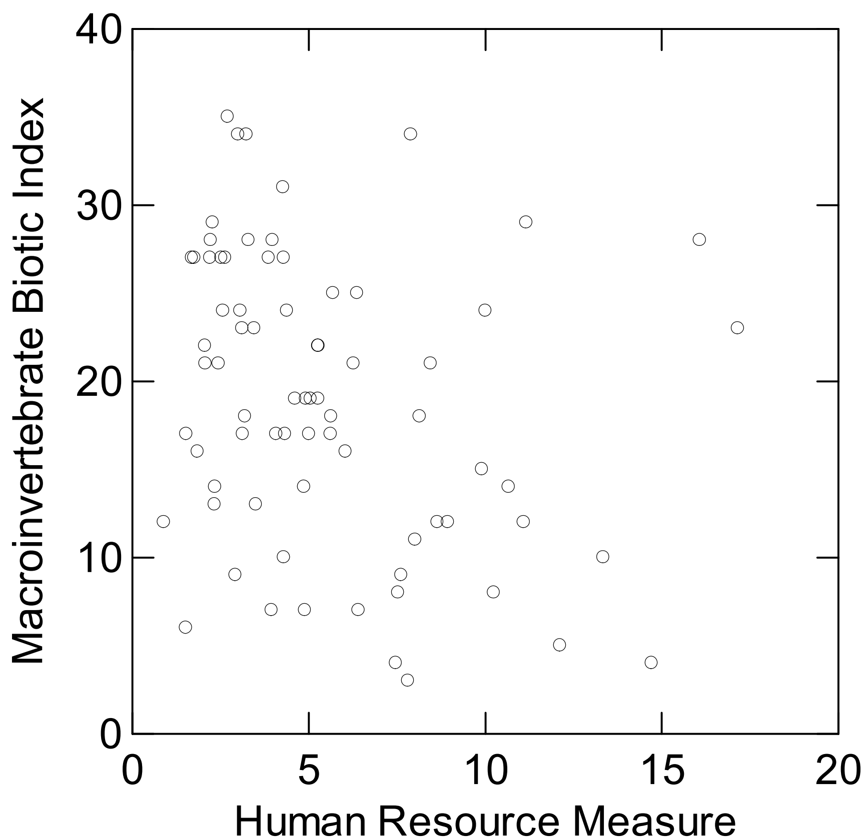
The presence of predators (in the form of crayfish, salamanders, minnows, and more complex fisheries including bullheads and sunfish) did not have any significant influence on the MBI. Box-plot comparisons of MBIs grouped by the type of predator present showed that agriculture and urban sites had consistently lower MBIs than reference wetlands (Fig. M4).

Figure M4. Macroinvertebrate biotic index scores within A) agriculture sites, K) reference kettles, P) reference prairies, and U) urban sites separated by the type of fish predator present. Predator codes are: F = complex fisheries, M = minnows only, and N = none observed.



The association between MBI scores and the surrogate human disturbance gradient was statistically significant based on linear regression analysis (p=0.002; N=80, R=0.341,adjusted r-squared =0.105). However, several anomalous outliers clouded the relationship (Fig M-5). This included five wetlands that had MBIs higher (i.e., better) than expected based on the measure environmental variable suspected to represent the degree of human impact. While these five wetlands (3 urban and 2 ag-impacted) did appear to be receiving moderate to high loadings of either chloride or nutrient runoff, some aspect of their current condition remains favorable as habitat for macroinvertebrates. Three of the five wetlands exhibited high concentrations of TKN-nitrogen and total phosphorus that may have resulted from the inclusion of duckweed or other plant material in the water sample (samples were not filtered prior to analysis). Consequently, in a small number of cases the nutrient concentrations may not have accurately portrayed human disturbance. The MBIs in the lower left corner of the plot (Fig M5) are of less concern in that other unmeasured habitat attributes or environmental pollutants may have resulted in a lower MBI than expected based on the impact gradient (i.e., other factors may have limited macroinvertebrates than chlorides and or nutrients).

Figure M5. Response of macroinvertebrate biotic index to the human resource measure.

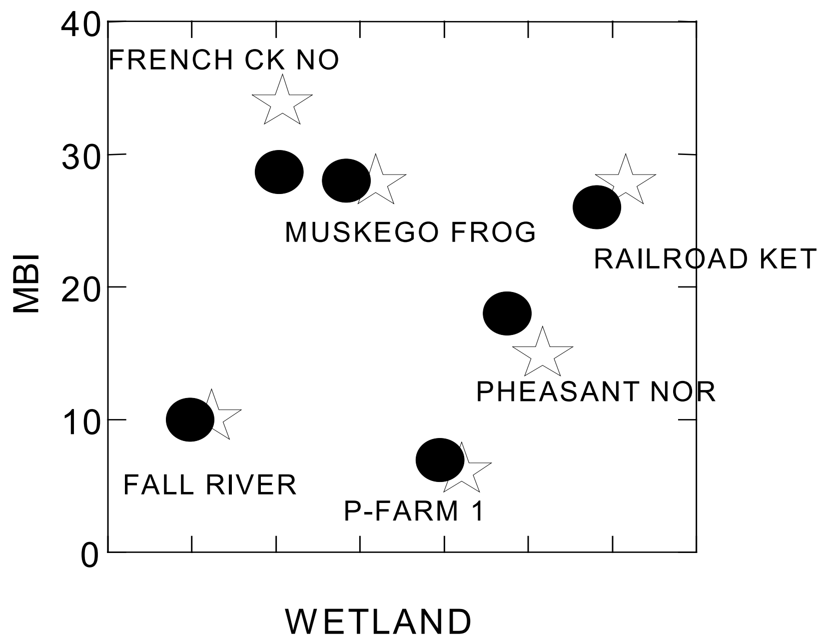


Replicate sampling:

Field replication:

The results from replicate field sampling were very encouraging. Three of the six replicate sets of MBI scores were within one biotic index unit and the greatest departure was five units (Figure M6). Five of the six replicate scores fell within the same qualitative rating class.

Figure M6. Field replicate MBI scores in six wetlands sampled in April 2000.



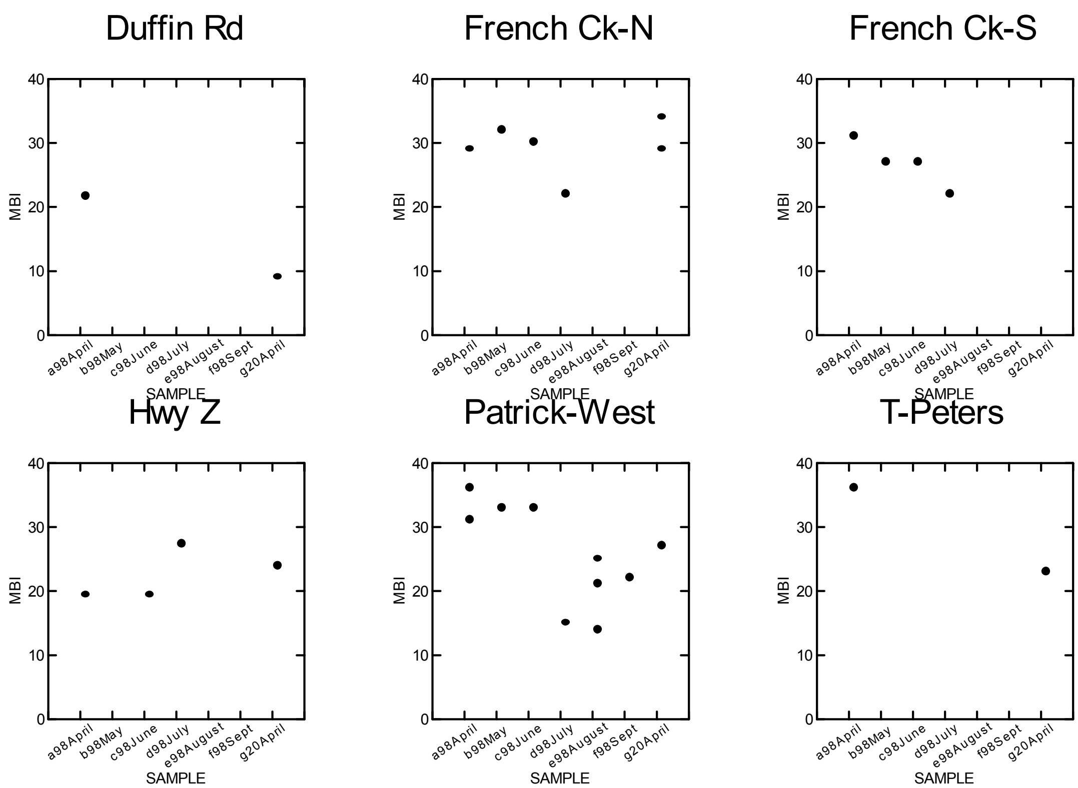
Seasonal and annual variability:

Six of the wetlands sampled during 2000 had been sampled previously during 1998 using the same field and laboratory protocols (Lillie 2000). We calculated MBI scores for each sample using the Modified MBI scoring system for long duration wetlands (Table M7). The performance of the MBI is presented in Figure M7, below. Each of the six wetlands had been sampled in April 1998 (with field replication in Patrick-West). The data labeled French Ck-N and French Ck-S represent samples collected from two interconnected basins that differed only very slightly in vegetative structure and depth. French Ck-N was also sampled during April 2000. Four wetlands were sampled periodically (roughly a month apart) during 1998 to address the issue of seasonal variation in the MBI. The series of three field 'replicates' shown for August 1998 in Patrick-West are not true replicates in the sense that they actually represent different areas of the same wetland (i.e., not composite samples representing the entire wetland).

Year-to-year changes (in this case change over two years) were considerable in 2 of the 5 wetlands sampled both in April 1998 and April 2000 (Fig. M7). MBI scores declined by more than one class in Duffin Rd. and T-Peters wetlands. There is a possibility that the severe drought that preceded sampling in April 2000 contributed to these declines, but we have no way of knowing for sure. The differences in the other three wetlands were not as severe.

Seasonally differences were judged as very severe based on the original evaluations and original MBI (Lillie 2000). This also was true using the Modified MBI for long duration wetlands as illustrated in Figure M7. MBI scores were somewhat similar in April-June, but scores decreased substantially in July. Consequently, we recommend that all macroinvertebrate sampling be restricted to the April-June period and preferably to the late April to early May (for Wisconsin). Sampling outside this window of time may produce unreliable results. Further research is required to document the responses of the individual metrics to natural events (e.g., long-term climatic variation, recovery following drought, introduction of exotics, etc.).

Figure M7. Seasonal and yearly changes in MBI scores within six wetlands. Sample dates coded 98 for 1998 and 20 for year 2000 samples.



Plant Community:

General Community Characteristics

Ninety-four plant taxa were recorded from the 74 wetlands surveyed (Table P1). Taxonomic richness averaged 8 to 9 taxa per wetland and ranged from none to a maximum of 26. The richness measure includes a mixture of taxonomic levels and excludes at least seven unidentified taxa. A list of the dozen most frequently occurring taxa is provided in Table P2, with a breakdown of each taxon's relative dominance (subjective categorization over the entire wetland) and average importance value (I.V.) within stands (i.e., average IV restricted to wetlands where it was found) and across all wetlands. Values reported for duckweeds, pondweeds, and *Carex* spp. represent a composite of multiple species within each taxonomic group. For example, the occurrence values for duckweeds represent the combined individual occurrence of *Lemna minor*, *Lemna trisulca*, and *Sprirodela polyrhiza*. Because more than one species of duckweed may have occurred in the same wetland, the total (87) exceeds the total number of wetlands in the sample. The average IVs represent the average of all of the IVs of individual species within each taxonomic group. Duckweed was frequently dominant or co-dominant (N=38) and ranked first in regards to IVs across all wetlands. Reed canary grass was the dominant emergent, being dominant or co-dominant in 24 wetlands. Pondweed, cat-tail, and less frequently, coontail were occasionally dominant and established relatively high importance values. *Carex* sp., arrowhead, and smartweed, although occurring quite frequently, were rarely dominant. Other taxa that were occasionally dominant included Chara (8), river bulrush (5), white water lily (4), watermeal (4), and water buttercup or crowfoot (4).

There was a significant relation between the subjective classification of a taxa and its average importance value (ANOVA, $R=0.575$, $r^2=0.330$, F-ratio=50.7, df=6, $p<0.001$) based on the quadrat surveys. Median importance values increased progressively from the lowest to highest dominance class (Table P3). The several outliers in the lower dominance classes represent instances where the particular taxon happened by chance to occur either more frequently or in greater relative cover in the 18 quadrats surveyed than in the entire wetland. Although not tested in this study, the data suggest that it may be possible to substitute or equate subjective classifications to relative importance values for computing an alternative plant biotic index score. If the subjective classification scores worked as well as the current WWPBI in differentiating among impacted and unimpacted wetlands, it would represent a considerable time cost savings in respect to sampling effort.

Table P1. Alphabetical list of plants occurring on study wetlands. Scientific name, common name, code abbreviations (used in appendix), and frequency of occurrence are shown for each taxon.

Taxon	Common Name	Abbreviation	Frequency
Acorus calamus	Sweet Flag	ACOCA	1
Algae	various filamentous algae	ALGAE	15
Alisma plantago-aquatica	Water-plantain	ALIPL	13
Alisma sp.	Water-plantain	ALISP	5
Asclepias sp.	Milkweed	ASCSP	2
Bidens sp.	Beggar’s ticks	BIDSP	2
Brasenia schreberi	Watershield	BRASC	1
Bromus inermis	Smooth brome grass	BROIN	2
Calamagrostis canadensis	Bluejoint grass	CALCA	3
Carex alopecoidea	Carex sedge	CARALP	1
Carex comosa	Bristly (or bottlebrush) sedge	CARCOO	4
Carex oligosperma	Carex sedge	CAROLS	5
Carex sp.	Carex sedge (unidentified)	CARSP	18
Carex straminea	Straw sedge	CARSTA	2
Carex stricta	Hummock sedge	CARSTC	2
Ceratophyllum demersum	Coontail	CERDE	17
Chara sp.	Chara or stonewort	CHARA	11
Cicuta bulbifera	Water-hemlock	CICBU	4
Dactylis glomerata	Orchard grass	DACGL	1
Dulichium arundinaceum	Pond sedge	DULAR	7
Eleocharis acicularis	Needle spike-rush	ELEAC	2
Eleocharis sp.	Spike-rush (unidentified)	ELESP	17
Elodea canadensis	Elodea or waterweed	ELOCA	2
Equisetum sp.	Horsetail	EQUSP	5
Eupatorium sp.	Joe-Pye weed or boneset	EUASPP	1
Galium sp.	Bedstraw	GALSP	1
Galium tinctorium	Stiff bedstraw	GALTI	6
Glyceria grandis	Reed mannagrass	GLYGR	6
Graminae	Grasses (unidentified)	GRASS	5
Hypericum borale	Northern St. John’s Wort	HYPBO	2
Impatiens sp.	Jewelweed	IMPSP	3
Iris sp.	Iris	IRISP	1
Iris versicolor	Blue flag iris	IRIVE	2
Juncus effusus	Common rush	JUNEF	1
Juncus sp.	Rush (unidentified)	JUNSP	1
Leersia oryzoides	Rice cut-grass	LEEOR	10
Lemna minor	Small or lesser duckweed	LEMMI	55
Lemna trisulca	Star or forked duckweed	LEMTR	19

Leonurus cardiaca	Motherwort	LEOCA	1
Lycopus uniflorus	Northern water-horehound	LYCUN	1
Lycopus sp.	Horehound or bugleweed	LYCUSP	1
Lycopus virginicus	Virginia water-horehound	LYCVI	1
Lythrum alatum	Winged loosestrife	LYTAL	1
Lythrum salicaria	Purple loosestrife	LYTSA	2
Mentha sp.	Mint (unidentified)	MENSPE	1
Myriophyllum spicatum	Eurasian water-milfoil	MYRSPI	1
Najas flexilis	Bushy pondweed	NAJFL	6
Nitella flexilis	Stonewort	NITFL	1
Nitella sp.	Stonewort	NITSP	2
Nuphar advena	Yellow water lily	NUPAD	2
Nymphaea odorata	Fragrant White water lily	NYMOD	6
Onoclea sensibilis	Sensitive fern	ONOSE	1
Osmunda cinnamomea	Cinnamon fern	OSMCI	1
Phalaris arundinacea	Reed canary grass	PHAAR	57
Phleum pratense	Timothy grass	PHLPR	1
Phragmites australis	Giant reed grass	PHRAU	1
Polygonum amphibium	Water knotweed (Smartweed)	POLAM	1
Polygonum sp.	Smartweed (unidentified)	POLOSP	27
Polygonum pensylvanicum	Pensylvania knotweed	POLPEN	1
Polygonum sagittatum	Arrow-leaved tearthumb	POLSAG	5
Potamogeton sp.	Pondweed (unidentified)	POTASP	28
Potamogeton crispus	Curly pondweed	POTCR	1
Potamogeton epihydrus	Ribbon-leaved pondweed	POTEP	1
Potamogeton filiformis	Thread-leaved pondweed	POTFI	1
Potamogeton foliosus	Leafy pondweed	POTFO	3
Potamogeton gramineus	Variable-leaved pondweed	POTGR	1
Potamogeton illinoensis	Illinois pondweed	POTIL	1
Potamogeton natans	Common ponsweed	POTNA	4
Potentilla palustris	Marsh cinquefoil	POTPA	1
*Potamogeton pectinatus**	Sago pondweed	POTPE	10
Potamogeton vaseyi	Vasey's pondweed	POTVA	1
Ranunculus longirostris	Stiff water-crowfoot	RANLO	2
Ranunculus spp.	Buttercup or crowfoot	RANSP	9
Ricciocarpus natans	Liverwort	RICCAR	2
Riccia fluitans	Liverwort	RICCI	8
Sagittaria latifolia	Common arrowhead	SAGLA	9
Sagittaria sp.	Arrowhead (unidentified)	SAGSP	12
Salix sp.	Willow	SALSP	11
Scirpus cyperinus	Woolgrass sedge	SCICYC	6
Scirpus fluviatilis	River bulrush	SCIFL	12
Scirpus sp.	Bulrush (unidentified)	SCISP	13
Scirpus validus	Great bulrush	SCIVA	3
Sium suave	Water parsnip	SIUSU	8
Solanum nigrum	Black nightshade	SOLNI	10

Sparganium sp.	Bur-reed	SPASP	5
Sphagnum spp.	Sphagnum mosses	SPHSA	4
Spirodela polyrhiza	Great duckweed	SPIPO	13
Thelypteris palustris	Marsh fern	THEPA	1
Triadenum fraseri	Bog St. John's wort	TRIFR	1
Triadenum virginicum	Marsh St. John's wort	TRIVI	1
Typha angustifolia	Narrow-leaved cat-tail	TYPAN	3
Typha latifolia	Common cat-tail	TYPLA	31
Utricularia sp.	Bladderwort	UTRSP	8
Wolffia sp.	Watermeal	WOLFI	14
Unknowns & others	Unidentified taxa	UNKNOW	7

* now known as *Stucknia pectinatus* (Crow & Hellquist 2000).

Table P2. Dominance and importance values of the 12 most commonly occurring taxa, listed in order of frequency of occurrence. Dominance codes are: 1 = rare, 2 = occasional, 3 = common, 4 = abundant, 5 = co-dominant, and 6 = clearly dominant.

Taxon	**Freq. Occ.**	**Subjective Dominance Classification "1"**	**"2"**	**"3"**	**"4"**	**"5"**	**"6"**	**Importance Values Stand***	**All**	**Rank**
Duckweed	87	20	14	10	15	17	11	0.216	0.254	1
Reed-canary	57	12	5	7	9	8	16	0.169	0.130	2
Pondweeds	57	16	16	7	9	4	5	0.170	0.131	3
Cat-tails	34	2	9	6	4	7	6	0.131	0.060	4
Carex spp.	32	15	7	5	1	4	0	0.045	0.021	6
Smartweeds	29	10	12	5	0	1	1	0.042	0.016	8
Arrowheads	21	10	5	6	0	0	0	0.045	0.013	10
Spike-rush	19	8	4	4	2	1	0	0.048	0.012	11
Plantain	18	10	7	0	0	0	1	0.032	0.008	12
Coontail	17	5	6	2	1	2	1	0.115	0.026	5
Bulrush	17	7	5	2	3	0	0	0.076	0.017	7
Grasses	15	5	5	0	2	3	0	0.063	0.013	9

* excludes wetlands where taxon did not occur.

Table P3. Summary descriptive statistics of importance values by subjective dominance classification. Data include all 621 occurrences on all 74 wetlands.

Statistic	**Subjective Dominance Classification**					
Importance Value	**“1”**	**“2”**	**“3”**	**“4”**	**“5”**	**“6”**
Number	228	126	75	65	59	68
Median	.019	.028	.074	.140	.196	.320
Mean	.052	.052	.131	.169	.200	.321
1 SE	.006	.006	.018	.018	.016	.028

Table P4. Impact coefficients* for selected macrophytes listed in order from highest to lowest median impact.

Taxon or Taxa	**Frequency**	**Impact Value**	
		Mean ∀ 1 S.E.	**Median**
River Bulrush	12	8.02 ∀ 1.49	6.96
Algae	15	6.89 ∀ 0.85	6.42
Watermeal	14	6.17 ∀ 1.08	5.45
Lemna minor	55	6.04 ∀ 0.52	5.02
Reed Canary Grass	57	5.56 ∀ 0.50	4.93
Coontail	17	5.78 ∀ 0.90	4.93
Water Plantain	18	5.28 ∀ 0.68	4.79
Bushy Pondweed	6	4.33 ∀ 0.44	4.76
Cat-tail	34	5.79 ∀ 0.63	4.62
Bulrush	17	4.80 ∀ 0.61	4.62
Sago Pondweed	10	4.54 ∀ 0.66	4.51
Pondweed	57	4.92 ∀ 0.40	4.39
Duckweed	87	5.56 ∀ 0.40	4.28
Chara	11	4.21 ∀ 0.77	4.28
Smartweed	29	5.06 ∀ 0.59	4.09
Spike-rush	19	4.10 ∀ 0.58	3.98
Arrowhead	21	5.43 ∀ 0.87	3.51
Spirodela polyrhiza	13	5.11 ∀ 0.89	3.51
Liverwort	10	5.34 ∀ 1.45	3.27
Carex spp.	32	4.32 ∀ 0.52	3.22
Lemna trisulca	19	4.47 ∀ 0.83	3.12
Bladderwort	8	3.13 ∀ 0.44	2.80
Rice cut-grass	10	3.07 ∀ 0.60	2.65
Water buttercup	11	2.72 ∀ 0.20	2.58
Woolgrass sedge	8	3.56 ∀ 0.97	2.27
White water lily	6	2.77 ∀ 0.55	2.22

* impact coefficient values equal the sum of $\log_{10}$chlorides, total nitrogen and twice total phosphorus concentrations.

The distribution pattern of taxa along the human disturbance gradient was used to estimate a taxon's tolerance to human disturbance. The mean impact coefficient (i.e., mean of impact values for each wetland where the taxon was found) represents the typical magnitude of the impact in wetlands where the taxon occurs. Consequently, those taxa with high impact coefficients are likely more tolerant to human impact than those taxa with low impact coefficients. Many taxa occurred too infrequently to derive a valid coefficient. Impact coefficients for selected taxa are provided in Table P4. The median impact value for the 74 wetlands in the study was 4.51 (Urban = 6.95; Agriculture = 5.63; Reference kettles = 3.20; Reference Prairies = 3.07). River bulrush exhibited the highest impact coefficient, and white water lily exhibited the lowest impact coefficient. Filamentous algae, water-meal, lesser duckweed (*Lemna minor*), and reed canary grass had relatively high coefficients, suggesting that they were tolerant of or otherwise received some benefit from the forms of human disturbance associated with nutrient and chloride inputs. Two other duckweeds, namely *Spirodela polyrhiza* and *Lemna trisulca*, had much lower coefficients, suggesting that they were more sensitive to human disturbance than *Lemna minor* was.

Some anomalies were also apparent. Woolgrass sedge (*Scirpus cyperinus*) and rice cut-grass (*Leersia oryzoides*) had much lower impact coefficients than might be expected based on their association with disturbance (Wilcox et al. 1985, Wardrop & Brooks 1998, review by Adamus & Brandt 1990). Large disparities between a taxon's mean and median coefficient or a large standard error about the mean may suggest a greater unreliability in the median impact coefficient for that particular taxon.

Review of Preliminary Plant Biotic Index Development

WISCONSIN WETLAND PLANT BIOTIC INDEX

> The WWPBI is a multimetric index based on nine plant metrics derived from transect data (18 quadrats) and is intended as a supplementary index to the other indices to rate, rank, and compare wetland biological condition.

We developed the Wisconsin Wetland Plant Biotic Index (WWPBI) applying the same procedures used in formulating the macroinvertebrate-based index. The WWPBI index is designed to serve as a tool for evaluating the biotic integrity of depression wetlands in Wisconsin. Although in the course of our investigations we identified many plants to the species level for research purposes, we believe that a practical tool for managers with limited botanical training should be based on easily identifiable taxa at a coarse taxonomic level. Consequently, for the most part, we lumped taxa at various taxonomic levels (e.g., family, genus) or structural groups (e.g., grass-like, emergents) for analysis. We did include those species that were of common occurrence and were fairly easy to identify in the field (e.g., reed canary grass, rice cut-grass, woolgrass, and lesser duckweed). We examined importance values (average of percent cover and frequency of occurrence) and percent cover for emergent, submergent, floating-leafed, and open water attributes as the response attribute. The number of total plant taxa (includes unidentified taxa) per wetland was included. Most managers will be able to identify or otherwise separate the plant taxa in the field with a minimal of background training.

We evaluated 24 plant community attributes representing the major taxa groups found in 104 wetland basins as candidate metrics (a complete list of attributes tested is provided in Lillie 2000, Table 9). The total plant taxa attribute was the only richness measure examined, and included both identified and unidentified specimens found in the combined quadrat and general basin surveys. The majority of attributes tested were sums of importance values for all species in each taxa group (e.g., Carex IV = sum of individual *Carex* species). The importance value represented the average of the percent cover and frequency of occurrence of each taxon in the 18 quadrats surveyed within the 0-60 cm zone (please refer to methods section for more details). We only considered those taxa groups that were of common occurrence and that were easily identifiable. While some rare taxa undoubtedly would serve as good indicators, incorrect identifications could lead to many difficulties in developing a simple, field-employed, plant index. Consequently, we tried to keep the index simple and easy to apply. Some taxa (e.g., *Calamagrotis canadensis* = Bluejoint grass) initially may be difficult to identify in the field, but with a small amount of training and with experience the investigator should be able to easily separate between look-alike species. We also examined four simple percentage attributes based on overall coverage of the major plant community types (e.g., emergents, submergents).

We selected nine plant community attributes as metrics (two metrics were combined as an adjustment for longer duration wetlands) for development of the Wisconsin Wetland Plant Biotic Index (WWPBI) based on their response to potential sources of human impact (Table P5). The index is comprised of one richness metric, seven importance value metrics, and one percentage metric. Four metrics, including the number of plant taxa, Carex IV, Bluejoint grass IV, and Good-IV, demonstrate good positive associations with "natural" or relatively least-disturbed situations. Because these metrics exhibit a positive association with least-disturbed situations, they are expected to decline with increased levels of human disturbance. The "Good-IV" was intended to serve as a composite measure of the more desirable plants in the wetland community. The "Good-IV" metric represents the sum of IVs of *Carex* spp., bladderworts, pondweeds, *Calamogrostis canadensis*, arrowheads, spikerushes, smartweeds, and horsetails. The preliminary version (Lillie 2000) of the "Good-IV" included rice-cut grass, *Leersia* spp., but because other studies suggest that this taxon is tolerant to siltation disturbance (Wardrop & Brooks 1998), we excluded it from the current index. This modification changed the scores of eight wetlands in the original data set, but it did not influence assignment of rating scores because most of the affected wetlands were not reference wetlands (i.e., not among the data used to set the rating scores).

The other three metrics, namely RCG-IV (reed canary grass), TYPHA-IV (cat-tails), and DUCK-IV (Lemnaceae), are good negative indicators and respond to human disturbance by increasing in impacted wetlands.

Because most of the original metrics (initial seven) relied predominantly on emergent taxa, the index was biased in favor of less permanent wetlands. More permanent basins tended to have fewer emergents (relative cover) and higher contributions by submergents and floating-leafed vegetation. In an attempt to counteract this apparent bias, we adjusted the WWPBI by including a composite metric that would account for the submergent and floating leafed plant communities (which become increasingly important in longer duration wetlands). After screening all candidate attributes we choose a composite metric consisting of the average of the POND-IV (pondweed) and % floating-leafed attributes. Pondweeds (includes several genera and species; some are also floating-leafed) commonly occur in deeper undisturbed wetlands, and the coverage of floating-leafed plants generally was higher on reference wetlands than disturbed wetlands. The addition of this combination metric allowed an “upward” adjustment ranging from 1 to 5 in the standard cumulative score. No score adjustment (i.e., no addition is made) is given for basins known beyond any doubt to have water hydroperiods too short to support a submergent plant community. In the case where a wetland’s hydroperiod is unknown and both pondweeds and floating-leafed plants are absent, one point is added to the WWPBI.

Several other attributes showed some promise as metrics but were dropped for various reasons. Of particular significance was the maximum importance value (highest individual importance value of all taxa at a site), which functioned as a pseudo dominance indicator. We hypothesized that high maximum dominance values would be associated with disturbed wetlands, but the data did not support this assumption. Several taxa, including sphagnum, spikerushes, and percent submergent, showed some promise but either were inconsistent or occurred too infrequently to permit their incorporation into the final index (See Lillie 2000 for further details).

The Wisconsin Wetland Plant Biological Integrity Index (WWPBI) consists of eight component metrics (Table P5). We assigned scores to each of the selected metrics using the trisection technique as described for the macroinvertebrate metrics. Scores based on the total plant taxa metric are dependent upon the ability of the field investigator to recognize that different species are present. Although it is not necessary to identify all specimens to species, it is important that all taxa present in a wetland are recorded (as unidentified species A, B, C., etc.) or collected and returned to the laboratory for further identification. Unfortunately, the ability to recognize that different taxa are present is limited by the experience or training of the field staff, and undoubtedly some taxa are probably overlooked in the process of conducting the cursory survey. It is likely that various sedge, grass, and pondweed species are often missed or overlooked. While it would be ideal to make extensive collections (followed by laboratory identifications), this index was designed to function at a coarse taxonomic level. Therefore, in most cases a family or genus level identification, accompanied by a note if more than one species is likely present, is adequate for this index.

In scoring importance value based metrics, we combined multiple species under a particular taxonomic grouping <u>after</u> calculating importance values for individual taxa. For example, if two or more *Carex* spp. were present, we calculated IVs for each species (as unidentified species #1, #2, etc.) present in the 18 quadrats and then combined (summed IVs) the data as *Carex* spp. This accounts for overlapping distributions (i.e., co-occurrences) of different species of the same genus or family within the 18 qudrats used in the survey.

The deep water community adjustment as presented herein boosts index scores of longer duration wetlands by 1 to 5 depending upon the coverage of pondweeds and other floating-leafed plants present. This adjustment is intended to compensate for an unintentional bias in a preliminary version of the WWPBI that favored shorter duration wetlands (which naturally have a greater predominance of emergent vegetation used in the metrics) by acknowledging that other metrics may indicate a healthy biotic index in longer duration wetlands. Among the longer duration wetlands in the developmental data set (wetlands sampled during 1998), the presence and relative importance of pondweeds and other floating-leafed vegetation (e.g., water lilies) demonstrated a negative response to human disturbance. Consequently, the two attributes were combined into a single metric that is applied to longer duration wetlands. A wetland known to have a hydroperiod of less than eight months (and contains no pondweed or floating-leafed taxa) receives no adjustment score to the final WWPBI. Wetlands of unknown hydroperiod that lack both pondweeds and other floating-leafed vegetation receive a deep water adjustment score of '1'.

WWPBI index scores may be need to be adjusted to compensate for natural differences in plant community composition within particular wetland types (e.g., sedge meadows, bogs, etc.) that occur among ecoregions or across the tension zone (Curtis 1959).

Table P5. Assignment of scores for the Wisconsin Wetland Plant Biotic Index.

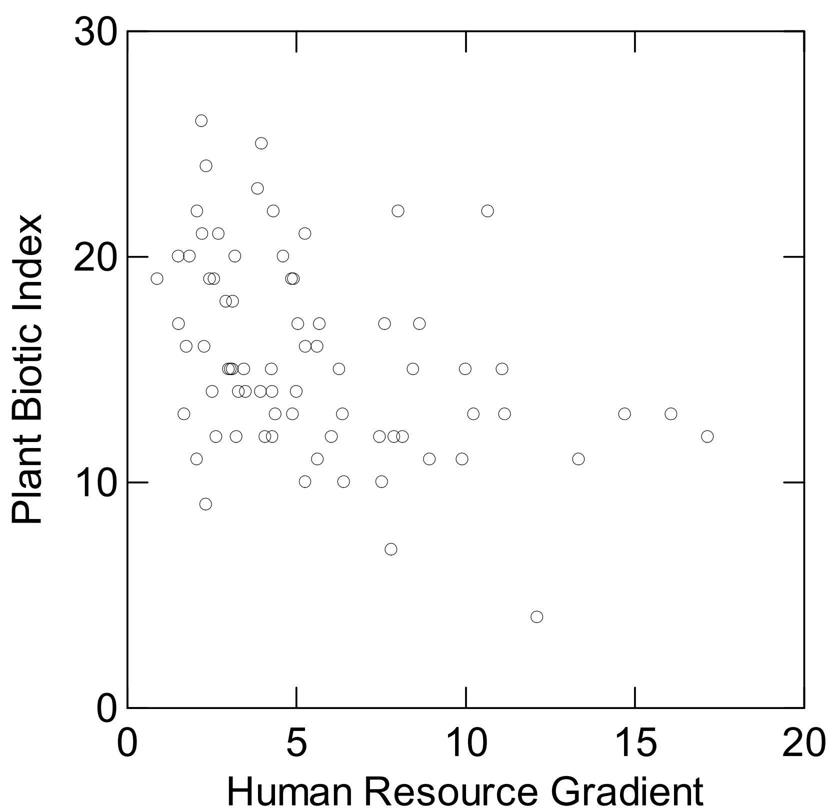
TAXA	**Attribute**	**Limitations**	**Response**	**Scores** 0	1	3	5	**Modifications**
Total Taxa	Count	Taxonomic resolution	Decrease	0-1	2 to 8	9 to 16	> 16	Future (?)
Carex	IV*	None	Decrease	0	< 0.1	0.1 to 0.36	>0.36	None
Reed Canary Grass	IV	None	Increase	>0.5	0.05 to 0.5	>0 to 0.05	0***	None
Cat-tail	IV	None	Increase	>0.25	0.03 to 0.25	> 0 to 0.03	0***	None
Duck-weed	IV	None	Increase	> 0.6	0.2 to 0.6	> 0 to 0.2	0	None
Bluejoint grass	IV	None	Decrease	-	0	> 0 to 0.05	> 0.05	None
Good**	IV	None	Decrease	0	> 0 to 0.3	0.3 to 0.6	> 0.6	None
Deep water Community Adjustment (+ 1 to 5 maximum)								
(PondIV + %Floating-leafed)/ 2								
Pond-weed	IV	> 7 months duration	Optimum	-	0	> 0 to 0.12 & > 0.4	0.12 to 0.4	None
Floating-leafed	Percent	> 7 months duration	Decrease	-	0	> 0 to 0.3	> 0.3	None

* IV represents Importance Values based on average or relative percent occurrence and relative cover.
** includes all ***Carex, Utricularia, Potamogeton, Calamogrostis, Sagittaria, Polygonum***, and ***Equisetum*** species.
*** If total taxa # 1 and if no emergents are present or is represented by an annual, then score as a 'zero'.

Performance of the Plant Biotic Index

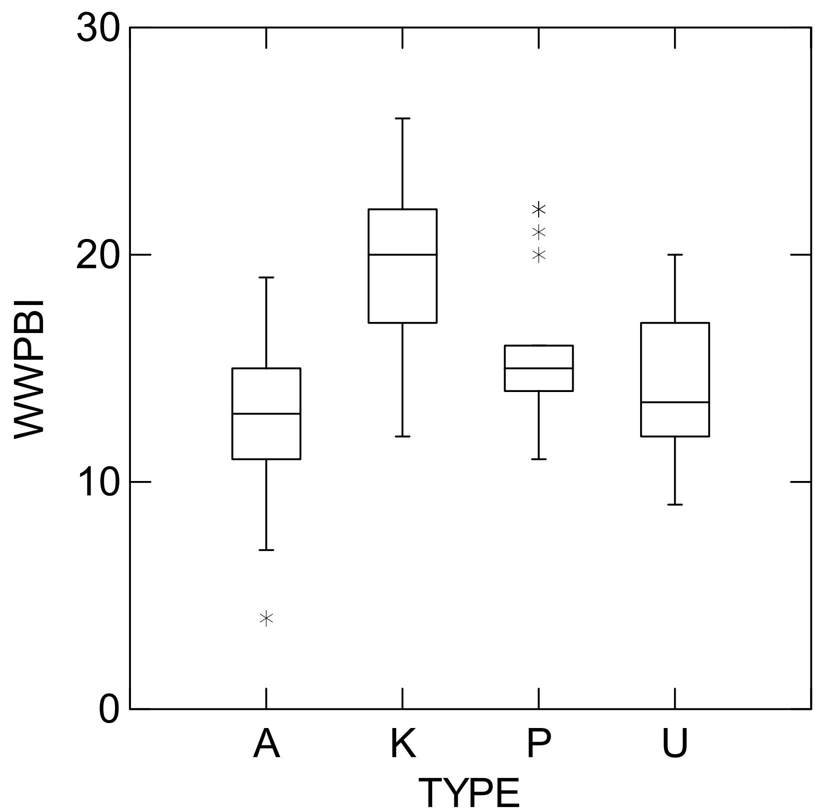
WWPBI scores for the 74 wetlands sampled during 2000 are provided in Appendix P, along with the component metric scores. WWPBI scores ranged from 4 in a highly impacted agricultural wetland to 26 (a rating of 'very good') in a forested reference kettle depression. Only four wetlands in the test data set achieved a quality rating of 'good' based on their WWPBI scores, whereas 15 wetlands in the developmental data set (i.e., wetlands sampled during 1998) rated as 'good' or better. WWPBI values were significantly correlated (inverse relation, $r^2 = 0.176$, $p<0.001$) with the human impact surrogate measures of nutrients and chlorides (Fig P1).

Figure P1. Response of plant biotic index to human resource gradient.



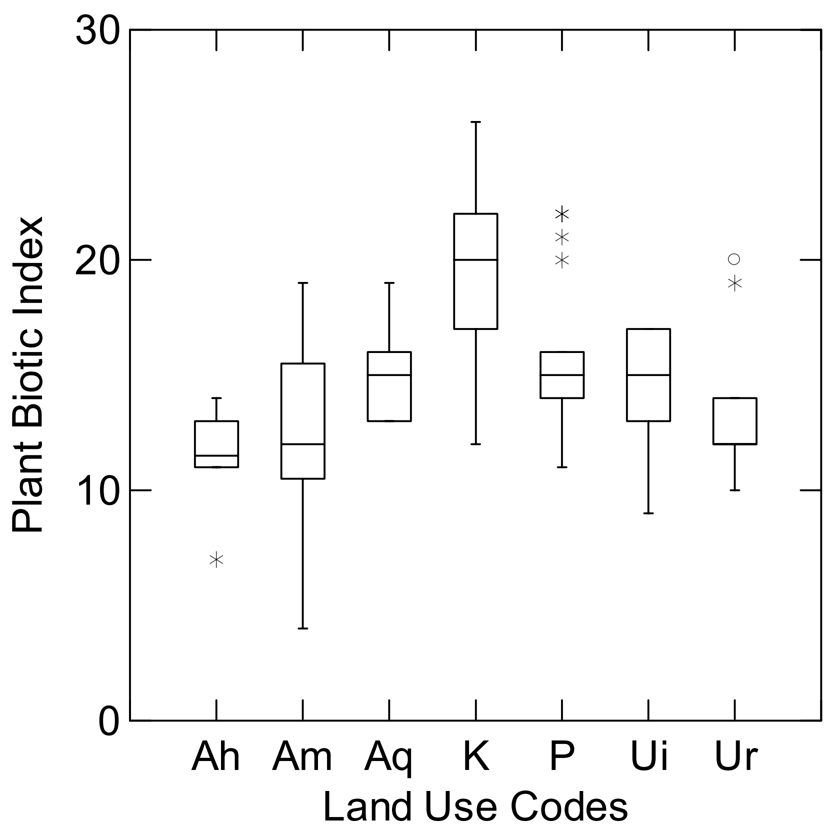
WWPBI values differed significantly (ANOVA, p<0.001) among basic land use types. WWPBI values in kettles were higher than all other wetland classes, including reference prairie wetlands (Tukey multiple comparisons p<0001, p<0.001, and p=0.022 for kettles versus agriculture, urban, and prairie, respectively). WWPBI scores in reference prairies were higher (albeit differences were not significant) than urban and agriculturally-impacted wetlands (Fig. P2).

Figure P2. Box plots of plant biotic index scores by wetland types.



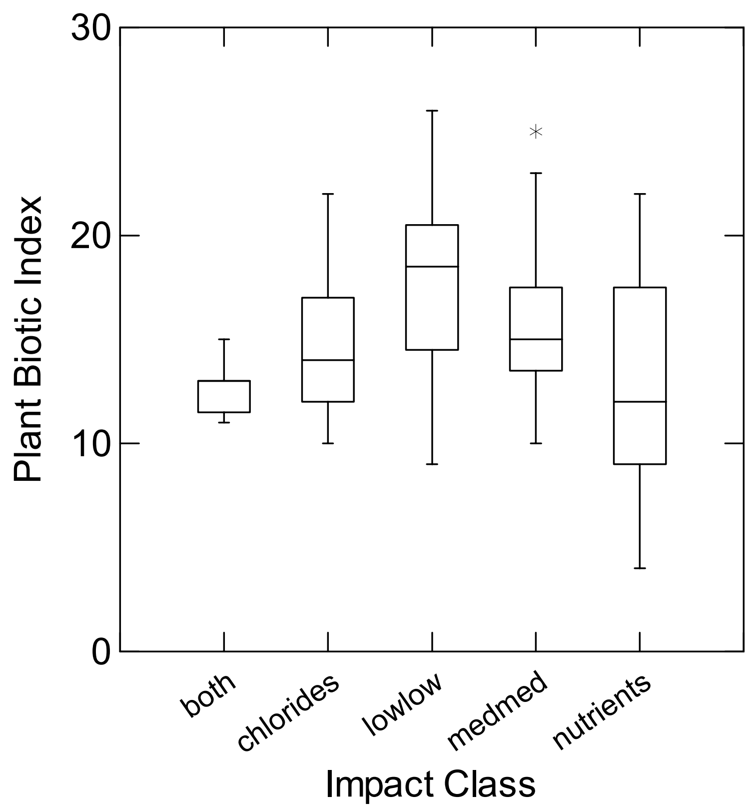
On a finer scale, WWPBI scores also differed significantly among the reference wetlands and the three agricultural impact classes and two urban classes (ANOVA based on ranks, $p<0.001$). A Dunn's pairwise comparison test indicated that reference kettles had significantly higher WWPBI scores than highly (Ah) and moderately (Am) agriculturally impacted and residential – urban (Ur) impacted wetlands (Fig P3).

Figure P3. Box plots of plant biotic index scores by land use classifications.



Comparisons among objective chloride-nutrient classes (Fig P4) revealed a significant impact on WWPBI scores (ANOVA based on ranks, p=0.002). Wetlands with both low nutrients and low chlorides had significantly higher WWPBI scores than both classes of wetlands with high nutrients ($p<0.05$). WWPBI scores in wetlands with only high chlorides (low or moderate nutrients) did not differ significantly from the least impacted wetlands. Consequently, the data suggests that the WWPBI is sensitive to nutrients and relatively unresponsive to high chloride inputs (or pollutants associated with high chloride inputs).

Figure P4. Box plots of plant biotic index scores by impact classification.



DIATOMS

General Characteristics of the Wetland Diatom Communities

A minimum of 300 and a maximum of 500 valves were counted for each sample. Samples from silty sites were diluted during processing to prevent diatoms from being obscured by particles on the microscope slide. This resulted in extremely low densities of diatoms, so only 300 valves were counted for these samples (B07, B10, B32, L06, and Z26).The diatom nomenclature used was that of Krammer and Lange-Bertalot (1991-1997), Patrick and Reimer (1966, 1975), and Hustedt (1930). Updated nomenclature from more recent literature was used when possible (see Appendix D-1 for nomenclature and references.) Counts were converted to proportions for use in subsequent metrics.

A total of 223 different diatom species and varieties were found in the 79 samples. Shannon-Wiener diversity ranged from a maximum of 3.3 at B17 (Bong 2, prairie site) to a minimum of 1.7 at B32 (Pabst Farm 3, agricultural site) (Table D-1). Species richness for the 72 sites with 500-valve counts ranged from 53 taxa at Z30 (Fall River, urban site) down to 19 taxa at Z13 (Mohr Road South, agricultural site). Richness for the 7 sites with 300-valve counts ranged from 41 taxa at L06 (Middleton Menards, urban site) down to 17 taxa at B32 (Pabst Farm 3, agricultural site). In order to compare richness between counts of 500 and 300 valves, the “Richness per Count” attribute was used. It equals the richness divided by the number of valves counted.

Table D-1. Descriptive statistics for community attributes. These are grouped by the number of valves counted and include the five replicates.

	500 valves; n = 72				300 valves; n = 7				All Counts; n = 79			
Community Attribute	Range	Median	Mean ± 1 SE	C.V.%	Range	Median	Mean ± 1 SE	C.V.%	Range	Median	Mean ± 1 SE	C.V.%
Diversity	1.9 to 3.3	2.8	2.8 ± 0.04	0.1	1.7 to 3.0	2.8	2.673 ± 0.175	0.2	1.7 to 3.3	2.8	2.7 ± 0.04	0.1
Richness	19 to 53	34	35 ± 0.9	0.2	17 to 41	28	30 ± 3.1	0.3				
Richness per count	0.04 to 0.1	0.07	0.07 ± 0.002	0.2	0.056 to 0.1	0.09	0.10 ± 0.01	0.3	0.04 to 0.1	0.7	0.07 ± 0.002	0.2

Biotic Index Development and Refinement.

Six kinds of metrics were evaluated for inclusion in the diatom index:

- Community metrics: diversity, richness adjusted for number of valves counted, and dominance measured as species present at greater than 10% in a sample (Charles 1999);
- Microhabitat preferences: various siltation indices, aerophils, and planktonic diatoms;
- Van Dam et al. (1994) metrics for pH, salinity, nitrogen uptake, oxygen requirements, saprobity, trophic state, and moisture;

- ▪Morphological guilds: filamentous and nonfilamentous eucentrics, araphids, monoraphids, cymbelloids, naviculoids, nitzschioids, eunotioids, epithemioids, and surirelloids (Molloy 1992);
- ▪Genera: all species and unknowns within a genus were combined for these metrics;
- ▪Species: species were included if they occurred in at least 10% of sites (Kelly & Whitton 1995). If varieties and subspecies of a taxon were also present, they were combined for an overall species metric.

In testing the metrics, proportion data were transformed using the arcsine square root transformation (Zar 1984). Log +1 transformations were used for water chemistry and environmental variables. Van Dam et al. (1994) metrics were not transformed.

Possible metrics were evaluated with regard to their correlation with environmental variables indicative of human impact, and for differentiation between wetland types, use classes, and developmental stages. Those with the most potential were evaluated for use in a multimetric index.

No clear patterns emerged for community metrics, whether compared among site types, different land uses including agricultural intensity and buffer width, developmental stages, or the human disturbance gradient (ANOVA; $p > 0.05$ for all). Some of the microhabitat metrics were significantly different among land use classifications but for others there were no distinct patterns or the data was so scarce as to be entirely comprised of outliers when analyzed with ANOVA. A few of the Van Dam et al. (1994) metrics had clear patterns of response to the human disturbance gradient and land uses, while others did not. Some of the morphological guild metrics were significantly different among land use classifications and by the human disturbance gradient, but they were correlated with the genera and species metrics so they were dropped in favor of metrics at higher taxonomic levels.

Different combinations of promising metrics were assembled and tested until a final combination was chosen that best delineated the responses of the diatom assemblage to different land uses and the human disturbance gradient. For all statistical tests, significance was set at $p \leq 0.05$. Differences in metrics and the multimetric index between various land use classifications were analyzed with ANOVA and Fisher's Least-Significant-Difference Test.

Metrics used in Multimetric diatom index:

The Wetland Diatom Biotic Index (WDBI) consists of six different metrics: the Van Dam et al. (1994) metrics for salinity and saprobity, aerophilic diatoms including difficult species, *Gomphonema* spp., *Nitzschia palea*, and *Stauroneis phoenicenteron*. The responses of the six metrics used in the WDBI to water chemistry, wetland basin attributes, and the human disturbance gradient (using the composite chemical index, or CCI, as a surrogate) are shown in Table D-2. Strong correlations between some of the metrics and environmental variables indicative of human impact may be concealed by interactions between the numerous environmental variables. Even if strong correlations were lacking, all six metrics were significantly different among land uses, impact classes, or developmental stages.

Van Dam et al. (1994) metric scores were calculated by multiplying the proportion of each species by its indicator value, summing for all species in a sample, and dividing by the number of valves counted.

The Van Dam et al. (1994) salinity classification (Halophil metric) was used to differentiate sites based on diatom preferences and tolerances for chloride levels. Diatoms preferring elevated salinity, such as *Craticula halophila* and *Ctenophora pulchella*, were rated higher than species intolerant of chlorides. These halophilous species indicate elevated salinity levels and conductivity. Increased conductivity and salinity in the habitat can be due to human impacts such as road salt runoff, or natural impacts such as evaporation in the water body (Johansen 1999). The Halophil metric was calculated without data for *Nitzschia incognita*. Numerous specimens of this taxon (up to 20% of valves counted at site L08) were found which resembled the figures in Krammer & Lange-Bertalot (1997). However, Van Dam et al. (1994) list *Nitzschia incognita* as a mesohalobous species occurring at 1000 to 5000 mg l^{-1} Cl. None of our wetlands had chloride concentrations this high. The lack of correspondence between the diatom's preferences and our water chemistry data suggest that this is not the correct taxonomical diagnosis. Thus, *Nitzschia incognita* was omitted from the calculations for the metric. The halophils in our samples were positively correlated with total Kjeldahl nitrogen (TKN) and negatively correlated with NO_2 - NO_3.

The Van Dam et al. (1994) saprobity classification (Saprophil metric)was intended to differentiate sites based on diatom tolerances to biodegradable organic matter and associated oxygen concentrations. Species such as *Navicula minima* and *Sellaphora seminulum* are more tolerant to organic matter input, and thus have higher indicator values than less tolerant species. The presence of tolerant species does not necessarily indicate presence of organic pollution; however the presence of intolerant species can be used to differentiate unpolluted sites from those with moderate or high amounts of organic pollution (Van Dam et al. 1994). Saprophils were positively correlated with silica and negatively correlated with calcium and alkalinity, but not any organic nutrients. However, if the most tolerant species are analyzed (indicator values of 4 or 5), they are positively correlated with total phosphorus, so this metric was retained.

Aerophilic diatoms were used as indicators for sites with reduced or "flashy" water residence times. This metric included the easily identified aerophils *Amphora submontanum*, *Diadesmis* spp., *Hantzschia* spp., *Luticola* spp., *Mayamaea* spp., *Nitzschia terrestris*, *Pinnularia borealis*, *Pinnularia obscura*, and *Stauroneis thermicola*. The aerophilic diatom metric also included the more difficult to identify *Navicula miniscula* var. *muralis*, *Navicula soehrensis* var. *hassiaca*, *Navicula tenelloides*, and *Nitzschia supralitorea*. If only the easily identified taxa are used, the metric still works to differentiate sites based on wetland type and to some degree for land use. However, differentiation is greater for sites based on land use if the "difficult" species are included. Aerophils were positively correlated with pH.

Genus and species metrics were based on the abundance of the taxa. *Gomphonema* tended to have higher numbers in reference sites and so this genus was used as an indicator for the reference condition. Some of the species we found, including the abundant *Gomphonema gracile*, prefer water with lower nutrient content (Patrick & Reimer 1975). *Gomphonema* was negatively correlated with total nitrogen, depth, and total nutrients. Of the six metrics, only *Gomphonema* trended toward a correlation with the human disturbance gradient ($p < 0.1$), and that was a negative correlation, as might be expected from the negative correlations with total nitrogen and nutrients.

Nitzschia palea is usually rated as one of the most pollution-tolerant diatoms. However, its presence does not always indicate organic pollution. It is common and is useful as an indicator of the silty habitats for which it is adapted. It was found at all but three of our sites

(B02, B07, B26) and those sites were kettle wetlands. It was also present at higher numbers in agricultural and urban sites than in kettles. *Nitzschia palea* was positively correlated with pH.

Stauroneis phoenicenteron was chosen because in initial examinations of the data it was present in only low numbers at urban sites, indicating possible sensitivity to an impact. Hall & Smol (1992) found that its total phosphorus optimum in British Columbia lakes was low, so we intended to use it as an indicator of low nutrient input. *Stauroneis phoenicenteron* was negatively correlated with NO_2 and NO_3, total nitrogen, depth, and total nutrients. However, *Stauroneis phoenicenteron* was also positively correlated with TKN and total phosphorus. It was present in mostly low numbers (mean 0.5%; range of 0 to 6.4% in counts), but it is a large diatom. If we had calculated diatom biomass we might have found that it was more important in our samples than is otherwise indicated by our count data.

Table D-2. Response of individual metrics to environmental attributes. This table shows the significance of non-parametric Spearman correlation coefficients between diatom index metrics and environmental attributes. Water chemistry data is (log+1) transformed, except for pH (untransformed), and basin size (arcsine square root). Excessive conductivity is an estimate of the amount of conductivity not predicted by alkalinity levels and is estimated as conductivity – 2(alkalinity) – chlorides (see Table M-4). CCI is the composite chemical index (a surrogate for the human disturbance gradient) and was calculated as the sum of total nitrogen + 2(total phosphorus) + log chlorides (see the Human Resource Disturbance Gradient section and Figure HD-1). Significance levels and the directions of the correlations are given.

Environmental Attribute	Van Dam Halophils	Van Dam Saprophils	Aerophils incl. difficult spp.	*Gomphonema* spp.	*Nitzschia palea*	*Stauroneis phoenicenteron*
Calcium	n.s.	$p \leq 0.005$; (-)	n.s.	n.s.	n.s.	n.s.
Chloride	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Color	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Conductivity	n.s.	n.s.	$p \leq 0.1$; (-)	n.s.	$p \leq 0.1$; (-)	n.s.
Excess Conductivity	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
pH	n.s.	$p \leq 0.1$; (+)	$p \leq 0.05$; (+)	n.s.	$p \leq 0.005$; (+)	n.s.
Alkalinity	n.s.	$p \leq 0.05$; (-)	n.s.	n.s.	n.s.	n.s.
NO_2 & NO_3	$p \leq 0.05$; (-)	$p \leq 0.1$; (-)	n.s.	$p \leq 0.1$; (-)	n.s.	$p \leq 0.01$; (-)
Total Kjeldahl Nitrogen	$p \leq 0.05$; (+)	n.s.	n.s.	$p \leq 0.005$; (+)	n.s.	$p \leq 0.05$; (-)
Total Phosphorus	$p \leq 0.1$; (-)	n.s.	n.s.	n.s.	n.s.	$p \leq 0.05$; (+)
Dissolved Silica	$p \leq 0.1$; (-)	$p \leq 0.05$; (+)	n.s.	n.s.	n.s.	n.s.
Total Nitrogen	n.s.	n.s.	n.s.	$p \leq 0.05$; (-)	n.s.	$p \leq 0.05$; (-)
Depth	n.s.	n.s.	n.s.	$p \leq 0.05$; (-)	n.s.	$p \leq 0.05$; (-)
Basin Size	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
CCI	n.s.	n.s.	n.s.	$p \leq 0.1$; (-)	n.s.	n.s.
Total Nutrients	$p \leq 0.1$; (+)	n.s.	n.s.	$p \leq 0.05$; (-)	n.s.	$p \leq 0.05$; (-)
TKN + P	$p \leq 0.05$; (+)	n.s.	n.s.	$p \leq 0.005$; (-)	n.s.	$p \leq 0.05$; (+)

There were significant differences among the different impact and development stage classifications for all of the individual metrics when analyzed with ANOVA (Table D-3). *A priori* types of wetlands (discussed in the Methods section) were different for all metrics, sub-classifications of land use and agricultural intensities were different for all but *Gomphonema*, and wetland developmental stages (discussed in Measures of the Human Disturbance Gradient section) were different for *Stauroneis phoenicenteron*.

Table D-3. Significant differences in ANOVA of metrics and different wetland categories. Nonsignificant differences are reported as "n.s." Wetland types are kettle, prairie, agricultural, and urban. Wetland types with sub-classifications include narrow and wide buffers for agricultural sites and industrial and residential designations for urban sites. Wetland types with ag intensities include low, medium, and high-intensity designations for agricultural sites and sub-classifications for urban sites. Wetland development stages are I, II, III, and IV, as shown in Figure 3 in the Human Resource Disturbance Gradient section.

Metric	**Wetland type**	**Wetland type with sub-classifications**	**Wetland type with ag intensities**	**Wetland development stage**
Halophils	$p \leq 0.05$	$p \leq 0.01$	$p \leq 0.05$	n.s.
Saprophils	$p \leq 0.05$	$p \leq 0.01$	$p \leq 0.01$	n.s.
Aerophils	$p \leq 0.005$	$p \leq 0.005$	$p \leq 0.005$	n.s.
Gomphonema spp.	$p \leq 0.05$	n.s	n.s.	n.s.
Nitzschia palea	$p \leq 0.01$	$p \leq 0.05$	$p \leq 0.05$	n.s.
Stauroneis phoenicenteron	$p \leq 0.005$	$p \leq 0.005$	$p \leq 0.01$	$p \leq 0.01$

Scoring Diatom Metrics

After a final set of metrics was selected, data from a randomly selected subset of 57 sites were used to set scoring ranges for the individual metrics. Data for each metric were tri-sectioned and values of 5, 3, or 1 were assigned, with 5 indicating better condition and 1 indicating worse condition. Halophils, Saprophils, Aerophils, and *Nitzschia palea* had inverse rating scales because they tended to increase with increasing impacts. Scoring ranges for each metric are given in Table D-4. These scoring criteria were then applied to the data for the remaining 22 sites, which included replicates. There was some discrepancy in scores between samples and replicates, so replicates were included to reduce the variability. The two sets of scored data were combined for use in subsequent steps.

Table D-4. Scoring ranges for individual metrics used in the WDBI. Trisectioning of data from 57 sites was used to set these ranges.

Metric **Score:**	**5**	**3**	**1**
Halophils (score)	≤ 1.68	1.68 – 1.83	≥ 1.84
Saprophils (score)	≤ 2.14	2.15 – 2.64	≥ 2.65
Aerophils (%)	$\leq 1\%$	1% – 5.6%	$\geq 5.7\%$
Gomphonema spp. (%)	$\geq 7.3\%$	3.4% – 7.2%	$\leq 3.3\%$
Nitzschia palea (%)	$\leq 3.6\%$	3.7% – 13.9%	$\geq 14\%$
Stauroneis phoenicenteron (%)	$\geq 0.3\%$	0.1% – 0.2%	0

Rating System for the WDBI

The scores assigned to each site for each metric were added together to create a single multimetric diatom index score for each site. The results for the reference sites (kettle and prairie sites) were used to assign narrative ratings to the array of WDBI scores. The narrative ratings were assigned by separating the scores into 10%, 25%, 50% (median), 75%, and 90% quantiles, as was done for the WMBI. Narrative ratings were assigned so that higher WDBI values indicated better wetland condition. The range of scores for each percentile and category are given in Table D-5. Figure D-1 shows the negative linear response of the WDBI to the human disturbance gradient. The linear regression is significant but very little of the variation in WDBI is explained by the human disturbance gradient ($R^2 = 0.098$).

Table D-5. Narrative rating system for WDBI scores based on distribution of scores within kettle and prairie wetlands.

Narrative rating	Range of WDBI scores
Excellent	≥ 29
Very Good	26-28.9
Good	22-25.9
Fair	19-21.9
Poor	12-18.9
Very Poor	≤ 11.9

Performance of the WDBI

The narrative rating system in Table D-5 was applied to the WDBI values for the non-reference agricultural and urban sites to sort the sites into the different classes of narrative ratings. The numbers of sites in each WDBI rating category, separated by types of wetlands, are shown in Table D-6.

Table D-6. Distribution of narrative WDBI ratings among the wetland types.

	Excellent	Very Good	Good	Fair	Poor	Very Poor	Sum	Excellent to Good	Poor to Very Poor
Kettle (ref.)	2	4	6	4	2	1	19	63%	16%
Prairie (ref.)	1		6	2	6	3	18	39%	50%
Agricultural			3	3	11	3	20	15%	70%
Urban	1		1	4	7	9	22	9%	73%

Ratings appear to correspond well with the types of wetlands. From 39% to 63% of reference sites were rated as Good to Excellent, while 16% to 50% of the sites were rated as Poor to Very Poor. The kettle sites tended to be rated better than the prairie sites. Of the impacted sites, only 9% to 15% of sites were rated as Good to Excellent, with 70% to 73% of sites ranked as Poor to Very Poor. Figure D-2 shows the distribution of WDBI scores by wetland type. Kettle sites were rated higher than the agricultural, prairie, and urban sites. Prairie sites were better than urban sites but not agricultural sites.

The number of sites in each WDBI narrative rating class, separated by impact classes, are given in Table D-7. Impact classes are designated by levels of total nutrients and chlorides and are discussed in the Human Resource Disturbance Gradient section and in Table H-1.

Table D-7. Distribution of qualitative WDBI ratings among objective impact classes.

Class	Nutrients	Chlorides	Excellent	Very Good	Good	Fair	Poor	Very Poor	Sum	Excellent to Good	Poor to Very Poor
1	Low	Low	2	2	7	4	5		20	55%	25%
2	Low/moderate	Low/moderate	1	2	5	4	7	6	25	32%	52%
3	Low/moderate	High			3	1	7	9	20	15%	80%
4	High	Low	1		1	1	3	1	7	29%	57%
5	High	High				3	4		7	0	57%

For impact classes, 74% of the sites that had either high nutrients or chlorides were rated as Poor or Very Poor. For sites with either high nutrients, high chlorides, or both, 70% of the sites received a Poor or Very Poor rating. High chlorides with low to moderate nutrients seemed to have the greatest effect on the rating; 80% of these sites received a rating of Poor or Very Poor. However, of sites with both low nutrients and low chlorides, 55% received Good to Excellent ratings. For sites with low or moderate nutrients and chlorides, 42% were rated as Good or better. However, 40% were rated as Poor or worse, which might be due to factors other than water chemistry, such as siltation.

Of the 27 sites with high chlorides, 74% were ranked as Poor or Very Poor and only 11% were ranked as good or better. Of the 14 sites with high nutrients, 57% were ranked as Poor or worse and only two sites, or 14%, were ranked as Good or better, and those were sites with low chloride levels. Of the seven sites that had both high nutrients and high chlorides, none of the sites ranked any better than Fair.

Figure D-3 shows a comparison of WDBI scores for the five different impact classes. Class 1 sites, with low nutrients and low chlorides, were rated higher than Classes 2, 3, and 5. Class 2 sites, with low to medium nutrient levels and low to medium chloride, were rated higher than Class 3 sites with low to medium nutrients and high chlorides.

The numbers of sites in each WDBI rating class, separated by impacted land use categories (as designated by agricultural row crop intensity and buffer widths, and urban land uses), are given in Table D-8.

Table D-8. Distribution of qualitative WDBI ratings among impacted land use categories. These ratings can be compared to those of the reference land use categories in Table D6.

Land Use	Excellent	Very Good	Good	Fair	Poor	Very Poor	Sum	Excellent to Good	Poor to Very Poor
Agriculture low			1	1	3	1	6	17%	67%
Agriculture medium			2	2	3	1	8	25%	50%
Agriculture high					5	1	6	0	100%
Agriculture narrow buffer					6	3	9	0	100%
Agriculture wide buffer			3	3	5		11	27%	46%
Urban industrial				3	3	5	11	0	73%
Urban residential	1		1	1	4	4	11	18%	73%

All of the high-intensity agricultural sites were ranked as Poor or Very Poor. Low and medium-intensity agricultural sites fared a little better, with 17% to 25% of sites, respectively, ranked as Good to Excellent. Low-intensity sites had slightly more sites ranked as Poor to Very Poor, at 67%, while medium-intensity agricultural sites had 50% of sites ranked as Poor to Very Poor.

For buffer width, all of the agricultural sites with narrow buffers were ranked as Poor to Very Poor. All but two of these were high intensity agriculture sites as well. Sites with wide buffer strips were rated better; 27% of these sites were rated as Good to Excellent and only 45% of sites were rated as Poor to Very Poor.

Of the 22 urban sites, two rated as Good to Excellent, and both of those were in residential areas. The majority, however, ranked as Poor to Very Poor. Industrial and residential sites both had 8 of 11, or 73% of sites, rated as Poor or Very Poor.

The ranges of WDBI scores, as compared among the different types of land use, are shown in Figure D-4. Figure D-4a shows the three intensities of agricultural land use and two kinds of urban use. Kettle sites ranked higher than low, medium, and high intensity agricultural sites, prairie sites, and urban industrial and residential sites. Prairie sites were rated higher than urban-industrial sites. There were no significant differences between WDBI scores for the three different agricultural intensities. Figure D-4b shows the effects of the two buffer widths in agricultural watersheds. Again, kettle sites were rated higher than narrow and wide buffer agricultural sites, prairie sites, and industrial and residential urban sites. Prairie sites were better than both urban-industrial sites and agricultural sites with narrow buffers. Agricultural sites with wide buffers were rated better than narrow buffer sites and urban-industrial sites. The wide buffer zones may be acting to slow runoff into the wetlands, preventing siltation and addition of excess nutrients from the watershed.

The numbers of sites in each WDBI rating class, separated by wetland development stages, are given in Table D-9. The four different development stages of wetlands are discussed in the Measures of the Human Disturbance Gradient section, and are illustrated there in Figure 3.

Table D-9. Distribution of qualitative WDBI ratings among wetland development stage categories.

Development Stage	Excellent	Very Good	Good	Fair	Poor	Very Poor	Sum	Excellent to Good	Poor to Very Poor
I	1		7	4	9	9	30	27%	60%
II	2	2	8	8	13	6	39	31%	49%
III					3	1	4	0	100%
IV	1	2	1	1	1		6	67%	17%

Between 26% and 30% of Stage I and II wetlands were rated as Good to Excellent. However, 60% of Stage I wetlands and nearly 50% of Stage II wetlands were rated as Poor to Very Poor. All of the Stage III wetlands rated as Poor or Very Poor. There were only 4 sites with this class of wetland. Since Stage III wetlands have high levels of fine organic substrate and low dissolved oxygen (see Figure 3), it would be expected that these sites would receive low ratings. Nearly 67% of type IV wetlands were rated as having Good to Excellent condition. All but one of these were kettle wetlands and these results are very similar to the distribution of scores for the types of wetlands, for which kettles had 64% of sites rated as Good or better and 16% of sites rated Poor to Very Poor.

The distribution of scores of the four development stages is shown in Figure D-5. Stage IV wetlands were rated as higher than Stage I, II, and III.

Summary

The WDBI was developed for wetlands in the Southeast Wisconsin Till Plain ecoregion (SWTP). It consisted of a set of six metrics: Halophils, Saprophils, Aerophils, *Gomphonema* spp., *Nitzschia palea*, and *Stauroneis phoenicenteron*. The WDBI performed well for detecting differences between land uses and variables indicative of human impacts. For the different wetland types, scores for kettles indicated better condition than agricultural and urban wetlands, and prairie wetlands were better than urban sites, but not agricultural sites. When separated by impact classes, the WDBI indicated that the sites with the best condition had low nutrients and chlorides, and quality deteriorated with increasing nutrients and chloride. High chlorides had the greatest impact on scores at sites with moderate nutrient levels. There were no significant differences among WDBI scores for the three levels of agricultural intensity or between industrial and residential urban sites. However, buffer widths did make a difference for the agricultural sites, where sites with wide buffers had better condition scores than sites with narrow buffers. The WDBI performed somewhat well for detecting differences among developmental stages of wetlands. Stage IV wetlands were rated better than the other classes, but this may be because most of our Stage IV wetlands were kettles, which tended to have higher scores. The WDBI could be further tested and refined by assessment of other alkaline wetlands in the SWTP in the future.

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Figure D-1.

Response of Wetland Diatom Biotic Index to Human Disturbance Gradient. The Human Disturbance Gradient was calculated as the sum of total nitrogen, twice total phosphorus, and log chlorides. The gradient was transformed using a (log + 1) transformation. The linear regression line is shown; little of the variation in the WDBI is explained by the human resource variable ($R^2 = 0.098$; $p = 0.005$). The different narrative ratings of the WDBI are delineated.

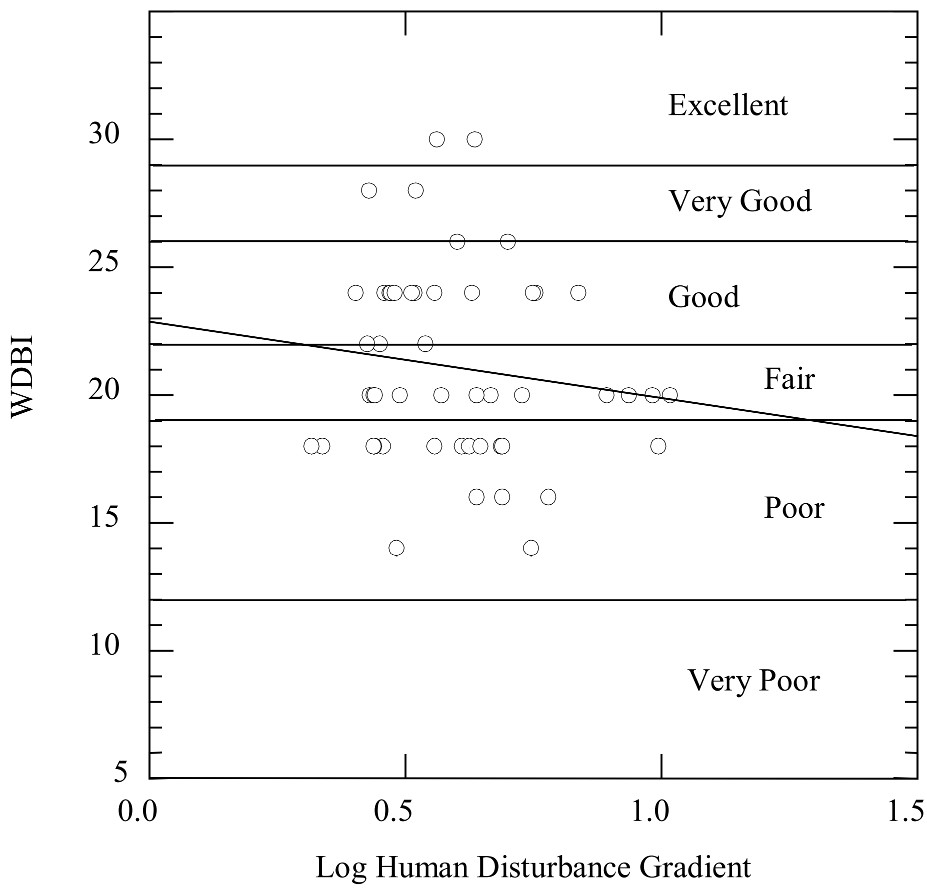
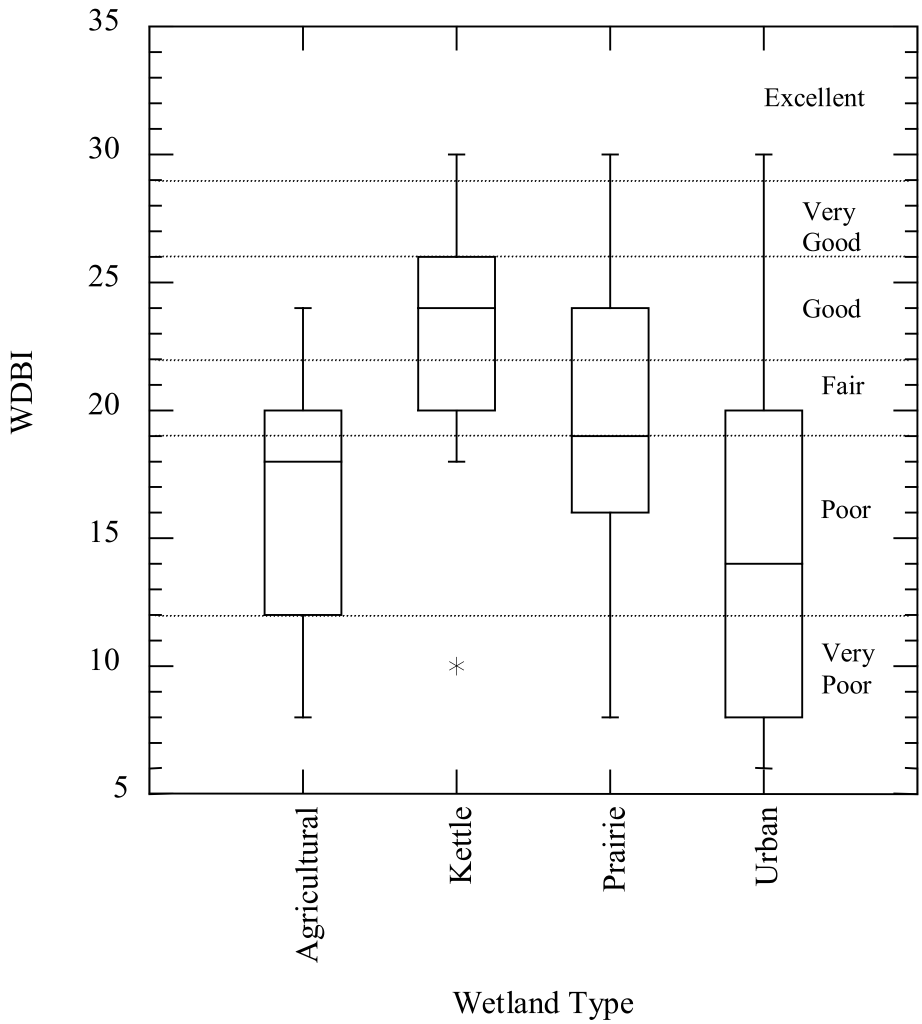


Figure D-2.

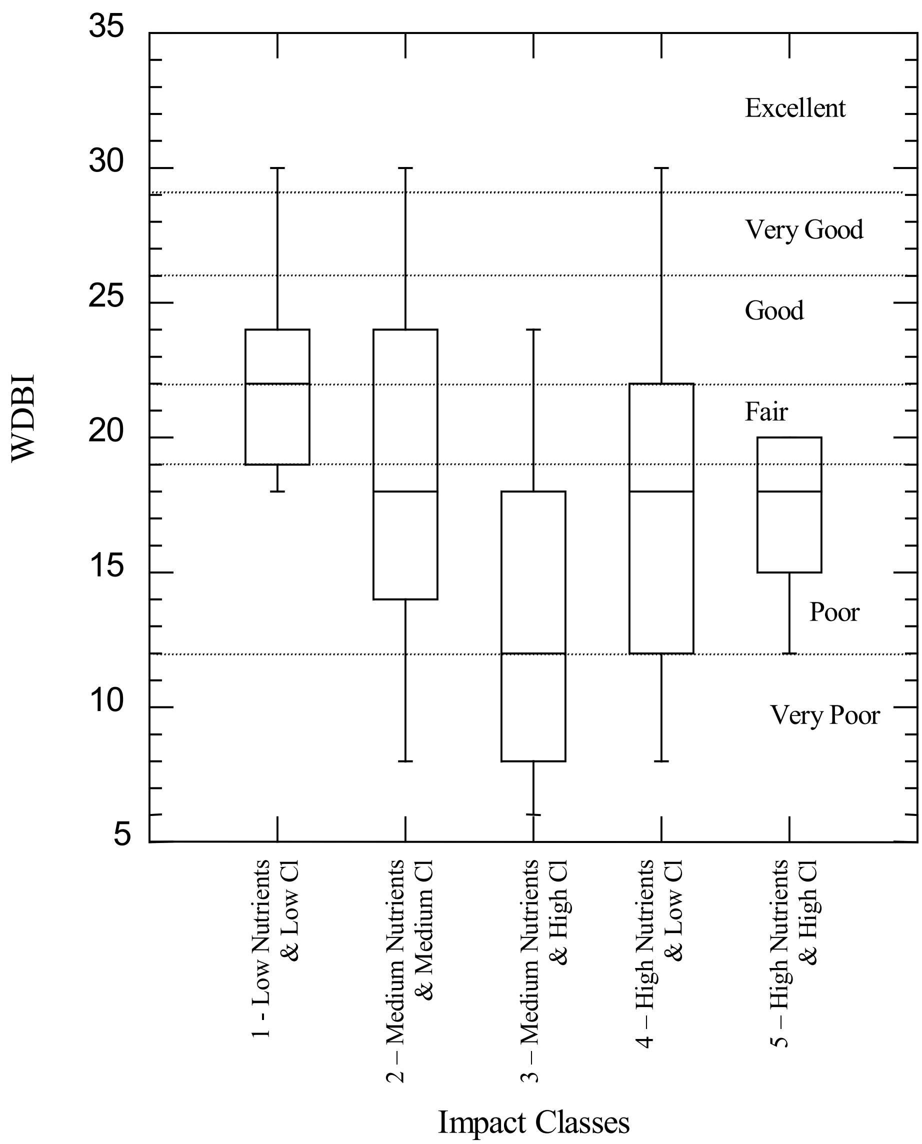
Wetland Diatom Biotic Index scores by wetland type. The outlier for kettle sites is B13, which had a score of 10. The highest possible WDBI score, indicating best condition, is 30. The lowest possible score is 6. Dotted lines show the cutoffs between narrative ratings for the WDBI.



Kettle > Agricultural, Prairie, Urban; Prairie > Urban.

Figure D-3.

Wetland Diatom Biotic Index scores differentiated by impact classes. Low nutrients are < 3 mg l^{-1}; medium nutrients are 3 – 5 mg l^{-1}; high nutrients are > 5 mg l^{-1}. Low chlorides are < 3 mg l^{-1}; medium chlorides are 3 – 10 mg l^{-1}; high chlorides are > 10 mg l^{-1}. The highest possible WDBI score, indicating best condition, is 30. The lowest possible score is 6. Dotted lines indicate cutoffs between the narrative ratings for the WDBI.

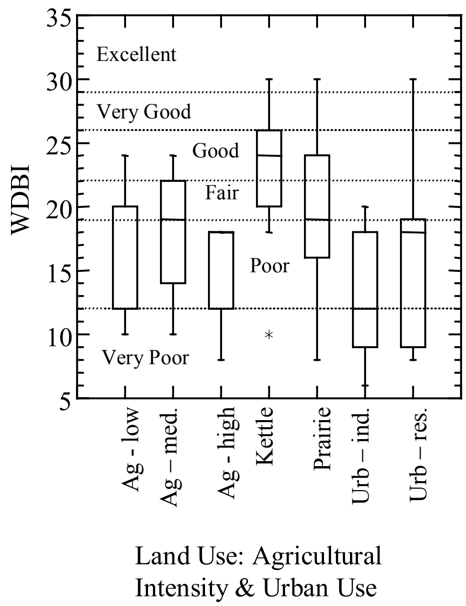


Class 1 > Classes 2, 3 & 5; Class 2 > Class 3.

Figure D-4.

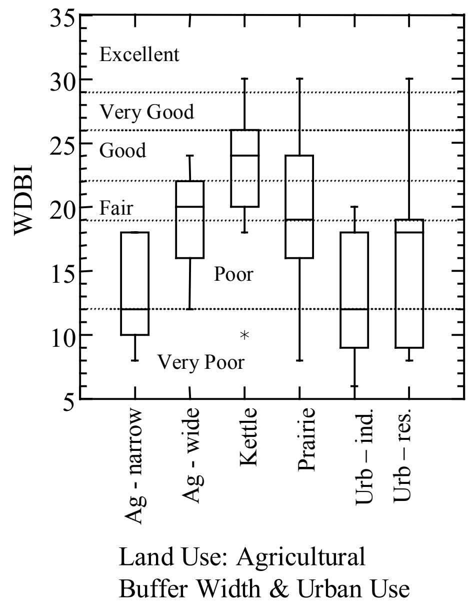
Wetland Diatom Biotic Index scores differentiated by land use subclasses. "Urb-in." are urban-industrial use sites. "Urb-res." are urban-residential sites. The outlier for kettle sites is B13, which had a score of 10. The highest possible WDBI score, indicating best condition, is 30. The lowest possible score is 6. In Figure D-4a, the median of the low-intensity agriculture data is 12, and the median of the high-intensity agriculture data is 18. The dotted lines show the cutoffs between the narrative ratings for the WDBI.

Fig. D-4a



Kettle > all;
Prairie > Urb-ind.

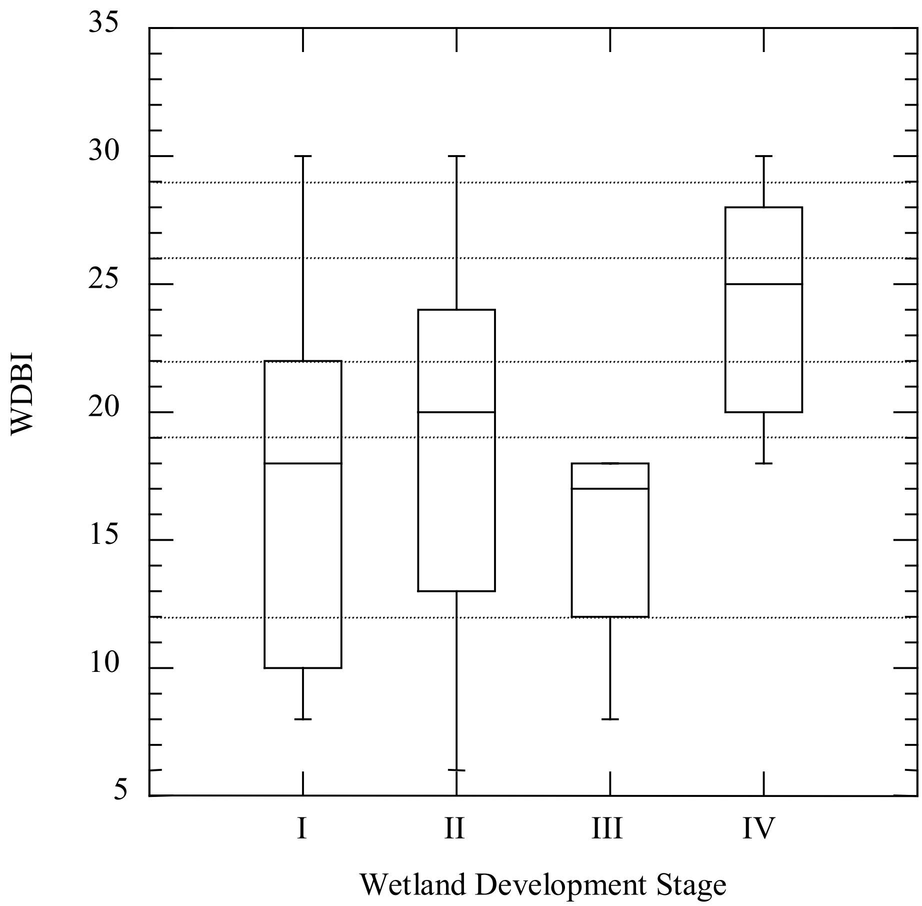
Fig. D-4b



Kettle > all;
Prairie > Ag-narrow, Urb-ind.;
Ag-wide > Ag-narrow, Urb-ind.

Figure D-5.

Diatom biotic index scores differentiated by wetland development stage. Stage IV wetlands scored significantly higher than the other three stages of wetlands. The highest possible WDBI score, indicating best condition, is 30. The lowest possible score is 6. The dotted lines show the cutoffs between narrative ratings for the WDBI.



Appendix D1. Diatom taxa with updated nomenclature

Nomenclature used	Authority	Former name or synonym
Achnanthidium exiguum var. *exiguum*	(A. Grunow) D.B. Czarnecki 1994	*Achnanthes exigua* var. *exigua*
Achnanthidium minutissima	(F.T. Kützing) D.B. Czarnecki 1994	*Achnanthes minutissimum*
Craticula accomoda	(F. Hustedt) D.G. Mann 1990	*Navicula accomoda*
Craticula cuspidata	(F.T. Kützing) D.G. Mann 1990	*Navicula cuspidata*
Craticula halophila	(A. Grunow) D.G. Mann 1990	*Navicula halophila*
Ctenophora pulchella	(A. Grunow) D.M. Williams & F.E. Round 1986	*Synedra pulchella*
Diadesmis confervacea	F.E. Round et al. 1990	*Navicula confervacea*
Encyonema minutum	(L. Hilse ex L. Rabenhorst) D.G. Mann 1990	*Cymbella minuta*
Encyonema silesiaca	(M. Bleisch ex L. Rabenhorst) D.G. Mann 1990	*Cymbella silesiaca*
Encyonopsis microcephala	(A. Grunow) K. Krammer 1997b	*Cymbella microcephala*
Fallacia pygmaea	(F.T. Kützing) A.J. Stickle & D.G. Mann 1990	*Navicula pygmaea*
Fistulifera pelliculosa	(A. Brébisson) H. Lange-Bertalot 1997	*Navicula pelliculosa*
Geissleria decussis	(E. Østrup) H. Lange-Bertalot & D. Metzeltin 1996	*Navicula decussis*
Gomphosphenia lingulatiforme	(H. Lange-Bertalot & Reichardt) H. Lange-Bertalot 1995	*Gomphonema clavatum* var. *lingulatiforme*
Hippodonta capitata var. *capitata*	(C.G. Ehrenberg) H. Lange-Bertalot, Witkowski, A. & D. Metzeltin 1996	*Navicula capitata* var. *capitata*
Hippodonta capitata var. *hungarica*	(C.G. Ehrenberg) H. Lange-Bertalot, Witkowski, A. & D. Metzeltin 1996	*Navicula capitata* var. *hungarica*
Lemnicola hungarica	F.E. Round & P.W. Basson 1997	*Achnanthes hungarica*
Luticola goeppertiana var. *goeppertiana*	(M. Bleisch in L. Rabenhorst) D.G. Mann 1990	*Navicula goeppertiana*
Luticola mutica var. *mutica*	(F.T. Kützing) D.G. Mann 1990	*Navicula mutica*
Luticola muticopsis	(H. Van Heurck) D.G. Mann 1990	*Navicula muticopsis*
Luticola nivalis	(C.G. Ehrenberg) D.G. Mann 1990	*Navicula nivalis*
Mayamaea atomus	(F.T. Kützing) H. Lange-Bertalot 1997	*Navicula atomus*
Navicella pusilla	(A. Grunow) K. Krammer 1997a	*Cymbella pusilla*
Navicula capitatoradiata	H. Germain 1981	*Navicula salinarum* var. *intermedia*
Navicula cryptocefalsa	H. Lange-Bertalot 1993	*Navicula cryptocephala*
Navicula schroeterii var. *escambia*	R. Patrick 1959	*Navicula symmetrica*
Navicula trivialis	H. Lange-Bertalot 1980	*Navicula lanceolata*
Pinnularia abaujensis var. *lacustris*	K.E. Camburn & D.F. Charles 2000	
Placoneis elginensis	(W. Gregory) E.J. Cox 1987	*Navicula elginensis*
Planothidium lanceolata	(A. Brébisson) L. Bukhtiyarova & F.E. Round 1996	*Achnanthes lanceolata*
Planothidium lanceolata ssp. *frequentissima*	(A. Brébisson) L. Bukhtiyarova & F.E. Round 1996	*Achnanthes lanceolata* var. *frequentissima*
Proschkinia bulnheimii	(A. Grunow) D.G. Mann 1990	*Navicula bulnheimii*
Pseudostaurosira brevistriata	(A. Grunow ex H. Van Heurck) D.M. Williams & F.E. Round 1988	*Fragilaria brevistriata*
Sellaphora americana	(C.G. Ehrenberg) D.G. Mann 1989	*Navicula americana*
Sellaphora laevissima	(F.T. Kützing) D.G. Mann 1989	*Navicula laevissima*
Sellaphora pupula	(F.T. Kützing) C. Mereschkowsky 1902	*Navicula pupula*
Sellaphora seminulum	(A. Grunow) D.G. Mann 1989	*Navicula seminulum*
Sellaphora vitabunda	(F. Hustedt) D.G. Mann 1989	*Navicula vitabunda*
Staurosira construens f. *construens*	(C.G. Ehrenberg) D.M. Williams & F.E. Round 1987	*Fragilara construens* f. *construens*
Staurosira elliptica	(J. Schumann) D.M. Williams & F.E. Round 1987	*Fragilaria elliptica*
Staurosirella pinnata	(C.G. Ehrenberg) D.M. Williams & F.E. Round 1987	*Fragilara pinnata*
Tryblionella hungarica	(A. Grunow) D.G. Mann 1990	*Nitzschia hungarica*

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Amphibians

Basic Findings -- Frog/toad calling summary:

Ten of the twelve anurans (frogs and toads) known to occur in Wisconsin were represented in the 74 wetlands surveyed (Table F1 and Appendix F). The mink frog is generally restricted to northern counties and was not expected to occur in the study area. The other anuran not found in this study was Blanchard's cricket frog, which is listed as State Endangered in Wisconsin. Generally, taxon richness increased from the first phenology (early spring) to second phenology (late spring) within individual wetlands. Chorus frogs and spring peepers were the most frequently heard species during the first phenology (Table F1). Spring peepers were present in 95% of wooded kettles, 56% of prairie wetlands, 45% of agriculture wetlands, and 17% of urban wetlands sampled (Table F2). Chorus frogs remained active and abundant during the second phenology and were joined by eastern tree frogs as the co-dominant anuran. Eastern tree frogs occurred in about 75% of the reference kettles and prairie wetlands, 50% of agriculture wetlands, and only 22% of urban wetlands.

Table F1. Summary list of frogs and toads heard calling during the first two phenologies in 2000.

Order: Anura	First	Second	Combined
Family: Bufonidae (Toads)			
Eastern American Toad – *Bufo americanus americanus*	5	25	30
Family: Hylidae (Tree frogs)			
Chorus Frog – *Pseudoacris triseriata triseriata*	40	44	84
Spring Peeper – *Hyla crucifer crucifer*	40	22	62
Cope's Grey Treefrog – *Hyla chrysoselis*	0	2	2
Eastern Gray Treefrog – *Hyla versicolor*	7	42	49
Family: Ranidae (True Frogs)			
Bullfrog – *Rana catesbeiana*	1	1	2
Green Frog – *Rana clamitans melanota*	0	10	10
Pickerel Frog – *Rana palustris*	0	1	1
Leopard Frog – *Rana pipiens*	18	8	26
Wood Frog – *Rana sylvatica*	5	4	9
Total – all species combined	116	159	275

Table F2. Distribution of anuran records by impact classification and phenology.

	Wetland Impact Class							
	K		P		A		U	
Phenology	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Family: Bufonidae (Toads)								
Eastern American Toad –								
Bufo americanus americanus	0	6	0	9	2	5	3	5
Family: Hylidae (Tree frogs)								
Chorus Frog –								
Pseudoacris triseriata triseriata	9	13	12	11	9	11	10	9
Spring Peeper –								
Hyla crucifer crucifer	18	12	10	4	9	6	3	0
Cope's Grey Treefrog –								
Hyla chrysoselis	0	0	0	0	0	0	0	2
Eastern Gray Treefrog –								
Hyla versicolor	6	14	0	14	1	10	0	4
Family: Ranidae (True Frogs)								
Bullfrog –								
Rana catesbeiana	0	0	1	1	0	0	0	0
Green Frog –								
Rana clamitans melanota	0	6	0	2	0	1	0	1
Pickerel Frog –								
Rana palustris	0	0	0	0	0	0	0	1
Leopard Frog –								
Rana pipiens	2	0	7	3	8	4	1	1
Wood Frog –								
Rana sylvatica	4	0	1	0	0	3	0	1
Total – all species combined	29	51	31	44	29	40	17	24

Total taxon richness differed significantly among impact classes during both phenologies (ANOVAs). Richness was significantly higher in reference kettles than in urban impacted wetlands in both the first ($p=0.012$) and second ($p=0.016$) phenologies. When taxon richness was calculated on the basis of combined occurrences during both phenologies, taxon richness was significantly higher in reference kettles than in both urban and agriculturally impacted wetlands. Richness in prairie reference wetlands was significantly higher than in urban impacted wetlands. None of the reference wetlands (kettle or prairie) had fewer than two taxa for the combined phenologies, while 55% of the urban impacted and 25% of the agriculturally impacted wetlands had less than two taxa. Likewise, a greater portion of reference wetlands had four or five taxa for the combined phenologies than the urban or agriculturally impacted wetlands. When the agricultural and urban wetlands were further subdivided by the degree or type of impact (i.e., heavy, moderate, or low agriculture and industrial or residential urban), the only detectable significant difference in taxon richness was between reference kettles and industrial-urban wetlands (K>U; $p=0.007$). Agricultural intensity and buffer width did not demonstrate a significant influence on anuran taxon richness ($p>0.05$). Smaller sample sizes in the more detailed analyses together with considerable amount of variability in the data contributed to the inability to detect more significant differences among impacts.

Aruran taxon richness was significantly correlated with several human impact gradient measures, most notably log-transformed chloride concentration (linear regression $r^2 = 0.304$, F-ratio=31.4, $p<0.001$). Taxon richness was significantly different among the five categorical impact classes ($p<0.001$). Richness was significantly higher in wetlands with low or moderate nutrients and chlorides than in wetlands with elevated chlorides or elevated nutrients ($p<0.05$).

Some species showed promise as disturbance indicator taxa. Spring peepers were consistently most common on the reference kettles and least common on the urban wetlands. Eastern tree frogs were most common on the reference wetlands, particularly during the second phenology. Eighty percent of the occurrences of green frogs were on reference wetlands during the second phenology. The wood frog was heard calling only on five reference wetlands during the first phenology, but was found at four impacted wetlands during the second phenology. Copes tree frogs (2 sites) and pickerel frogs (1 site) were heard only at urban or agriculture wetlands during the second phenology. The impacted wetlands harboring these last three mentioned species occurred appear to represent the ‘best’ protected wetlands within each of these two impacted classes.

Frog Biotic Index Development:

The results of this preliminary investigation suggest that the anuran community may serve as an additional component of a suite of ecological indicators in assessing the health of Wisconsin wetlands. After evaluating the distribution of individual species among the various a priori wetland classes and the correlation of species with surrogate measures representing the human resource gradient, we derived a simple multimetric index comprised of three metrics (Table F3). The first metric represents the total number of different taxa observed or heard during both field visits. The second metric represents the sum of the combined counts during the two visits. This latter metric is a measure of the consistency or stability of the anuran community within an individual wetland across the two periods. A wetland in which several species are heard in both periods may score higher than a wetland which has a greater overall richness but where the observations were primarily restricted to one of the two periods. The third metric is the sum of the occurrences of five taxa that appear to occur more frequently in reference wetlands. The list includes spring peepers, toads, eastern tree frogs, green frogs, and wood frogs. Some occurrences are restricted to either the early or late periods (see Table F3). The frog biotic index (FBI) equals the sum of the three individual metric scores. The FBI ranges from 0 to 15, and the frog biotic index scale does not compare directly with either the macroinvertebrate or plant indices.

Table F3. The Frog Biotic Index with scoring system.

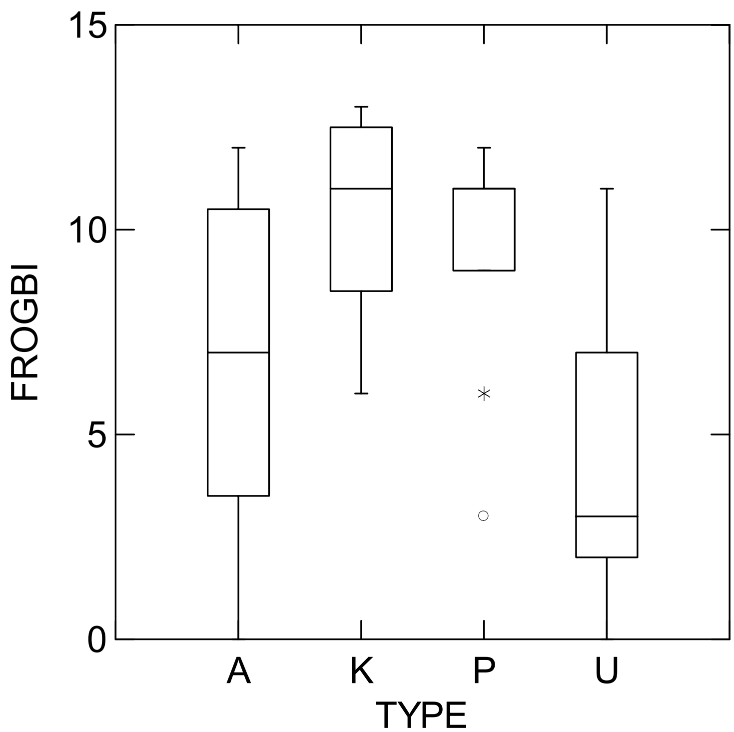
TAXON	**Attribute**	**Limitations**	**Response**	**Scores** **0**	**1**	**3**	**5**	**Modifications**
All taxa	Total Richness	weather	Decrease	0	1	2	3 or more	None
All taxa	Sum of individual richness during both periods	weather	Decrease	0	1 - 2	3 - 4	5 or more	None
Indicator taxa *	Sum of individual presences **	Weather	Decrease	Score ranges from 0 to 5 based on sum of five taxa present.				None

*Taxa include spring peeper, toads, eastern tree frogs, green frogs, and wood frog.
** Green frog during 2nd phenology; Wood frog during 1st phenology; others either phenology.

Frog Biotic Index Performance:

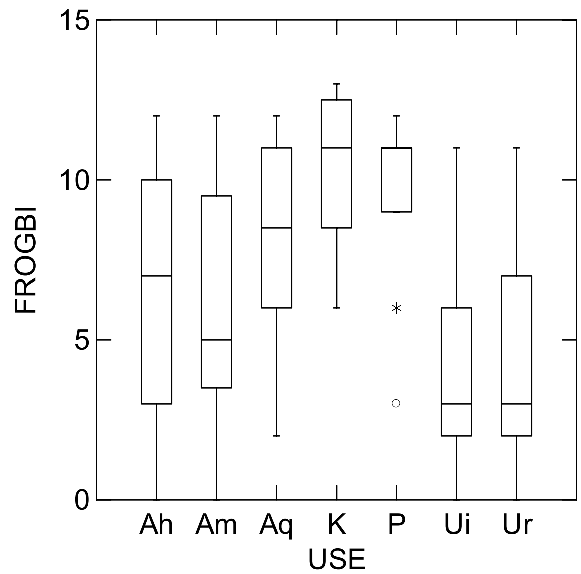
The FBI scores differed significantly (ANOVA results in Table F4) between reference and impacted wetlands (Fig. F1). FBI scores in kettles were significantly higher than in agriculturally impacted wetlands and urban impacted wetlands. FBI scores in prairie reference wetlands were significantly higher than in urban wetlands and marginally higher than agriculture wetlands.

Figure F1. Box plots of frog biotic index by wetland type.



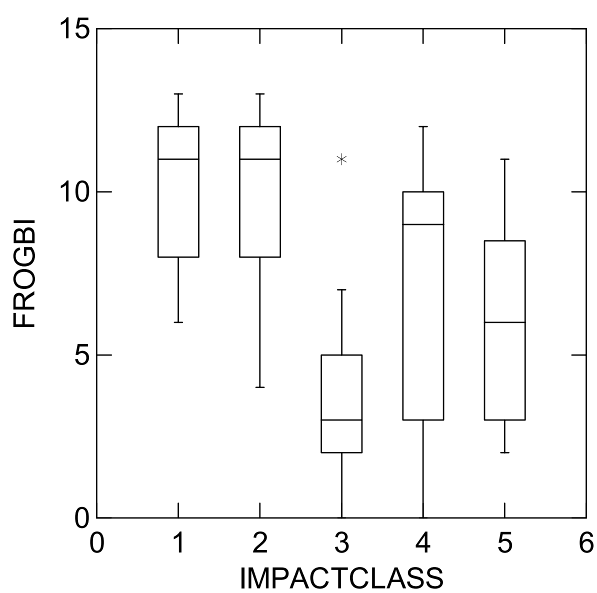
Significant differences remained after separating the agriculturally and urban impacted wetlands into their respective subclasses (Fig. F2 and Table F4; ANOVA, df=6, F-ratio=6.177, $p<0.001$). FBI scores were significantly higher in reference kettles than in industrial ($p<0.001$) and residential ($p=0.001$) urban wetlands, and FBI scores in reference prairie wetlands were significantly higher than in industrial ($p=0.009$) and residential ($p=0.025$) wetlands. FBI scores in agriculturally impacted wetlands were only marginally different from reference wetlands. The only statistically significant difference detected was that reference kettles had higher FBI scores than moderately impacted agriculture wetlands ($p=0.049$). Despite this finding, the mean FBI values in each of the three agriculturally impacted groups was lower than the 25 percentile for the two reference groups. The reduced ability of the FBI to detect a significant difference between the other two agriculture groups (Ah and Aq) and the reference groups was in part due to the smaller sample sizes and the occurrence of a couple high FBI scores in each group.

Figure F2. Box plots of frog biotic index scores by wetland use classification.



The FBI differed significantly by impact class ($p < 0.001$; Fig. F3). Wetlands classed as high chlorides (Class III) or high nutrients and chlorides (Class V) had lower FBIs than wetlands with low to moderate concentrations of chlorides or nutrients (all $p < 0.05$).

Figure F3. Box plots of frog biotic index by impact classification.



Regression and correlation analysis between the various environmental attributes (including the composite human resource gradient) suggested that the FBI was responding primarily to chlorides (negative correlation $p<0.001$ with $r^2 = 0.43$). A plot of the relationship clearly illustrates a strong negative linear response within each of the four wetland classes (Fig. F4). Despite the fact that the FBI was not statistically correlated with either TP, TN, or combined nutrients (all n.s., $p>0.05$), the FBI remained inversely correlated with the human resource measure (i.e., log-transformed Impact2 – negative correlation, $p<0.001$, $r^2=0.18$).

Figure F4. Response of frog biotic index to log-transformed chloride concentrations. Legend codes are A=agriculture, K=reference kettles, P=reference prairies, and U=urban. The ovals represent

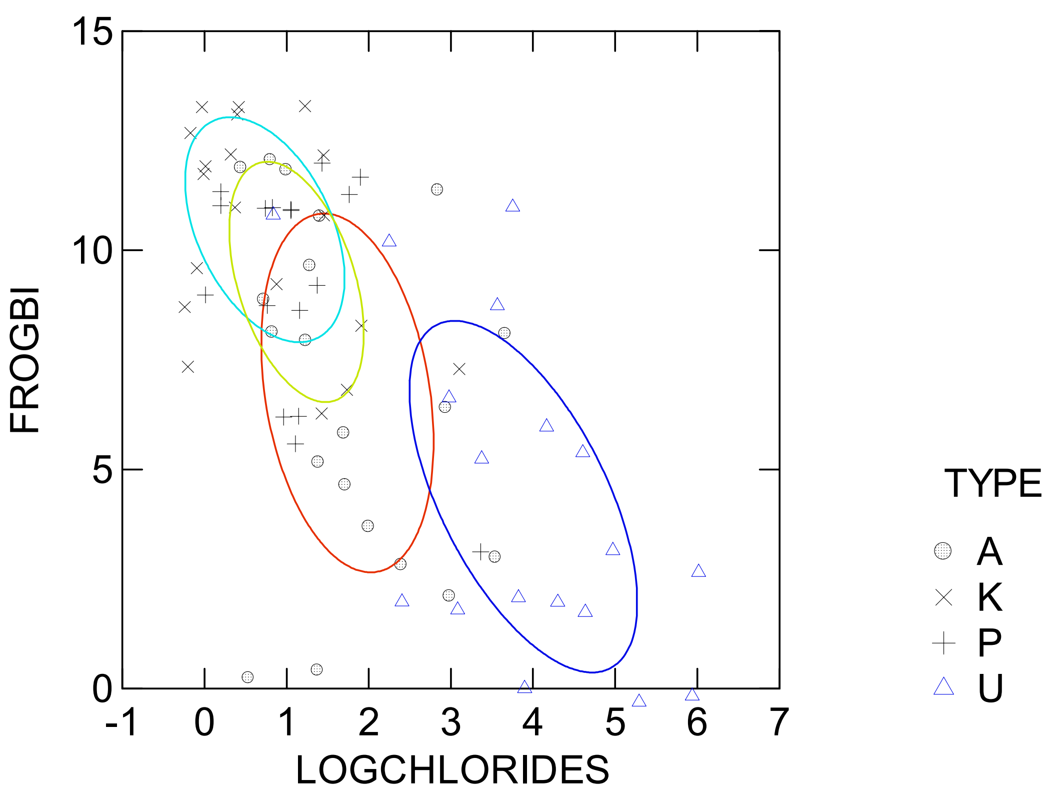


Table F4. Results of ANOVA testing on FrogBI with Bonferroni paired comparisons.

Anova Test	df	F-ratio	p-value	Significant Bonferroni comparisons	
Type (A,K,P,U)	3	12.179	<0.001		
				K > A	p=0.005
				K > U	p<0.001
				P > U	p<0.001
Land Use (Ah, Am, Aq, K,P, Ui, and Ur)	6	6.177	<0.001		
				K > Am	p=0.049
				K > Ui	p<0.001
				K > Ur	p=0.001
				P > Ui	p=0.009
				P > Ur	p=0.025

Small Mammals:

Basic Findings

Summary data:

A total of 466 small mammals were captured using a combination of three types of snap traps during the study period (Table SM1), representing the collections from a total of 2655 trap-nights on 81 wetlands (tally includes specimens captured during replicate sampling on seven wetlands). An additional 15 specimens, including 1 snake, 4 birds, and 10 frogs, were also collected. A summary list of captures for each wetland is provided in Appendix SM.

The large number of small mammals captured represents a relatively high trap success rate of 18.1% for all trap types combined. Trap success rate roughly equates to catch-per-unit-effort, or in this case, the number of mammals captured per trap set out per night. The 708 'sprung – no captures' (TableSM1) represent traps that were triggered but failed to capture an organism. The sprung traps included both undisturbed (triggered but not otherwise moved) and disturbed traps (moved from original position). Possible causes for these 'non-captures' include weak triggers, heavy rain, insects, small frogs, larger animals able to pull free, and targeted small mammals that escaped capture. A separate manuscript is being prepared comparing the trapping efficiencies among the three different traps employed in this study (Bautz In Prep.).

Ten species of mammals were recorded overall (Table SM2, and Appendix D). Almost two-thirds of captures were represented by two species, the meadow vole and white-footed mouse. The short-tailed shrew, meadow jumping mouse, and masked shrew also were found relatively frequently. The single Southern red-backed vole collected from Rocky-Run West, a reference kettle in Columbia County, was an unexpected surprise because this species generally occurs only in the northern two-thirds of the state, where it is associated with mature forests and large, well decayed woody debris on the forest floor. The single specimen of arctic shrew was collected at Schoenberg marsh, a prairie reference site in Columbia County (and a restored wetland), on September 28-29, 2000 during replication sampling. This collection was an unusual find because arctic shrews are uncommon along the southern edge of their range (the collection represents a new county record for Wisconsin).

Total small mammal abundance (i.e., as estimated by captures) ranged from 0-16 among the 74 wetlands (Table SM3). The greatest number of specimens taken at any one site (16) was from the Buethin Road wetland, an agriculturally impacted site in northwestern Dane County. The median number of captures among the four wetland types ranged between 4 and 5 specimens per trapnight, and the mean number of captures ranged between 4.72 specimens (urban wetlands) to 6.15 specimens (agriculture wetlands). No significant differences in average abundance (either among individual taxa or totals) were detected among the four wetland types (ANOVAs, all comparisons $p>0.05$), although the high number of masked shrew in reference kettles was nearly significant ($p=0.052$).

Small mammal taxon richness ranged from 0 to 5 (Table SM4), with a maximum richness of 5 recorded at the Hwy-Z kettle, a reference kettle in the Southern Unit of the Kettle Moraine State Forest. No small mammals were found on only two wetlands, P-Farm #1 (an agriculture site) and Baxter Park (an urban site). Taxon richness did not differ substantially among the four wetland types (ANOVA, df=3, F-ratio = 1.595, $p=0.198$).

The occurrence patterns of the small mammals generally were not related to the type of wetland in which they occurred. Chi-squared analysis suggested that the distributions of the three most commonly occurring taxa, including the short-tailed shrew, whitefooted mouse, and meadow vole, were not influenced by human impacts (all comparisons $p>0.05$). Among the less commonly occurring taxa (insufficient numbers to test using Chi-squared analysis), the distribution of two taxa may be related to impact. This includes the masked shrew, which was found on 11 of the 36 reference sites but only 2 of 38 impacted sites, and the house mouse, which was found only on 9 impacted sites (8 agriculture and 1 urban). The meadow jumping mouse tended to occur most frequently at agriculture sites; however, the difference in distribution among the four wetland types was not significant (Chi-squared, $p=0.072$).

Table SM1. Summary of trap collection efforts for 81 wetlands in Southeastern Wisconsin during 2000. Data includes frogs, birds, and one snake. Captures exclusive of replicates are provided in parentheses.

Trap measure	Museum specials	Standard Mouse traps	Rat Traps	Totals
Trap effort (=trapnights)	1215	1200	240	2655
Undisturbed	604	716	134	1454
Sprung – no captures	366	263	79	708
Missing	6	6	0	12
Captures	231 (204)	218 (197)	27 (26)	476 (427)
Capture rate per trapnight	19.0%	18.2%	11.3%	17.9%

Table SM2. Summary of small mammal trapping efforts by wetland type during summer 2000. Data include captures during late summer replicates on 7 wetlands. Data exclude birds and others.

Wetland Type (N=samples)	Shorttail shrew	Masked Shrew	Arctic Shrew	Whitefooted Mouse	Meadow Vole	Redbacked Vole	Medow Jumping Mouse	Flying Squirrel	Eastern Chipmunk	House Mouse	Totals
Agriculture (22)	23	6	1	37	47	0	13	0	2	8	137
Kettles (23)	14	11	0	47	49	1	5	0	3	0	130
Prairie (18)	21	3	0	16	59	0	9	0	1	0	109
Urban (18)	8	1	0	35	37	0	2	1	0	1	85
Combined (81)	66	21	1	135	192	1	29	1	6	9	461

Table SM3. Descriptive statistics for total small mammal captures (i.e., catch-per-unit-effort) by impact classification (replicates and non-mammals are excluded).

Attribute	Agriculture	Kettles	Prairie	Urban
N = samples	20	19	17	18
Range	0-16	1-11	3-12	0-12
Median	4.5	4	5	4.5
Mean	6.15	5.16	6.00	4.72
95% CI	4.16-8.14	3.77-6.54	4.53-7.47	3.21-6.24
SE	0.95	0.66	0.69	0.72
CV	0.692	0.557	0.475	0.645

Table SM4. Descriptive statistics for small mammal taxon richness by impact class (replicates and non-mammals are excluded).

Attribute	Agriculture	Kettles	Prairie	Urban
N = samples	20	19	17	18
Range	0-4	1-5	1-4	0-4
Median	2.5	3.0	2.0	2.0
Mean	2.55	2.63	2.35	1.94
95% CI	2.06-3.04	2.07-3.19	1.91-2.80	1.42-2.47
SE	0.24	0.27	0.21	0.25
CV	0.412	0.443	0.366	0.543

Development of Index

We found an insufficient number of candidate metrics among the several attributes of the small mammal communities tested to develop a multimetric index for Wisconsin wetlands. Neither total capture rates nor taxon richness appeared to be related to the human disturbance gradient as suggested by the a priori classification of wetlands into impacted and reference sites. Distribution patterns and relative abundances of the more commonly occurring small mammal taxa were not statistically correlated with impact. The presence/absence of some of the less frequently occurring taxa may have potential indicator value (e.g., masked shrew, arctic shrew, red-backed vole), but additional study will be required to substantiate their habitat associations and sensitivities to human impacts.

We suspect that a number of factors interfered with our ability to identify suitable metrics among the attributes of the small mammal community. We may need to address modifications in sampling design to account for mammal home range size, dispersal pathways, seasonal fluctuations and diel movement issues (e.g., migration?), to name just a few. Observations may need to be made at the landscape level rather than at a single point location. Part of the problem was that the size of the wetlands varied greatly and animals could have moved back and forth among wetlands. It was not possible in this study to control for the proximity to neighboring wetlands (of better or worse quality), wooded areas, or other favorable habitats. Consequently, small mammals collected at some impacted wetlands may have wandered in from neighboring wetlands of much better habitat quality. The presence of natural corridors among wetlands and the quality (dependent upon age, depth of duff layer, shading/cooling from trees, etc.) of habitat (as opposed to the areal estimation of quantity by percent cover of various land use types) were not addressed in this study. These factors and many more may have contributed to our inability to detect potential metrics. We believe that many of these issues may be addressed with modifications in sampling design and strategy; hence a workable solution to the problem is possible. Small mammals offer the potential as useful indicators of ecological condition. However, it is probable that a more extensive sampling effort will be required to identify indicator taxa and/or community metrics.

Zooplankton:

Note: Much of the data provided in this section are presented in a paper in preparation (Dodson and Lillie, In Prep.) and therefore will be only summarized here.

General:

We identified a sum of 59 taxa from the 74 wetlands sampled (Dodson and Lillie, In Prep.). The list included 25 branchipods (including the clam shrimp *Lynceus brachyurus*), 22 copepods, 9 ostracods, 1 rotifer, and 2 insects. Quantitative counts were not conducted except for *Daphnia pulex*, of which 8,384 specimens were identified as to male or female.

The majority of taxa occurred relatively infrequently with 31 of the 59 taxa being restricted to fewer than 5 sites. Of the remaining 28 taxa, only four taxa occurred in more than 50% of the samples. This short list included the branchipods *Ceriodaphnia* spp., *Daphnia pulex*, *Chydorus brevilabris*, and *Simocephalus vetulus* – all of which occurred across all four wetland classes. *Eucyclops agilis* and *Acanthocyclops robustus* were the two most frequently encountered copepods (48% and 40%, respectively). *Cyclocypris* sp (31%) was the most common ostracod, and the phantom midge, *Chaoborus* spp., occurred in 32% of the samples.

Twenty taxa were limited in their distribution to only one of the four wetland land use classes (e.g., agriculture, urban, wooded kettle, or open prairie). Fifteen taxa were unique to the combined reference wetlands and nine taxa were restricted to impacted wetlands (Table Z1). Although the four wetland classes contained approximately an equal number of total taxa (34-39 taxa composited across all wetlands within class), a great many of the taxa present in either of the two reference wetland classes were altogether absent from the impacted wetlands (Table Z1). Only one taxon, *Sinobosmina freyi*, was present in both impacted classes and absent from both reference classes.

Table Z1. Taxonomic summaries within wetland land use classes.

Wetland Class	Number sites	Total taxa – composite within class	Unique taxa – taxa restricted to class **	Number of Taxa present in reference classes but absent in this class
Agriculture	20	34	5	20
Kettles	19	37*	4	N.A.
Prairie	17	39*	7	N.A.
Urban	18	35	4	18

* 48 taxa pooled among kettles and prairies

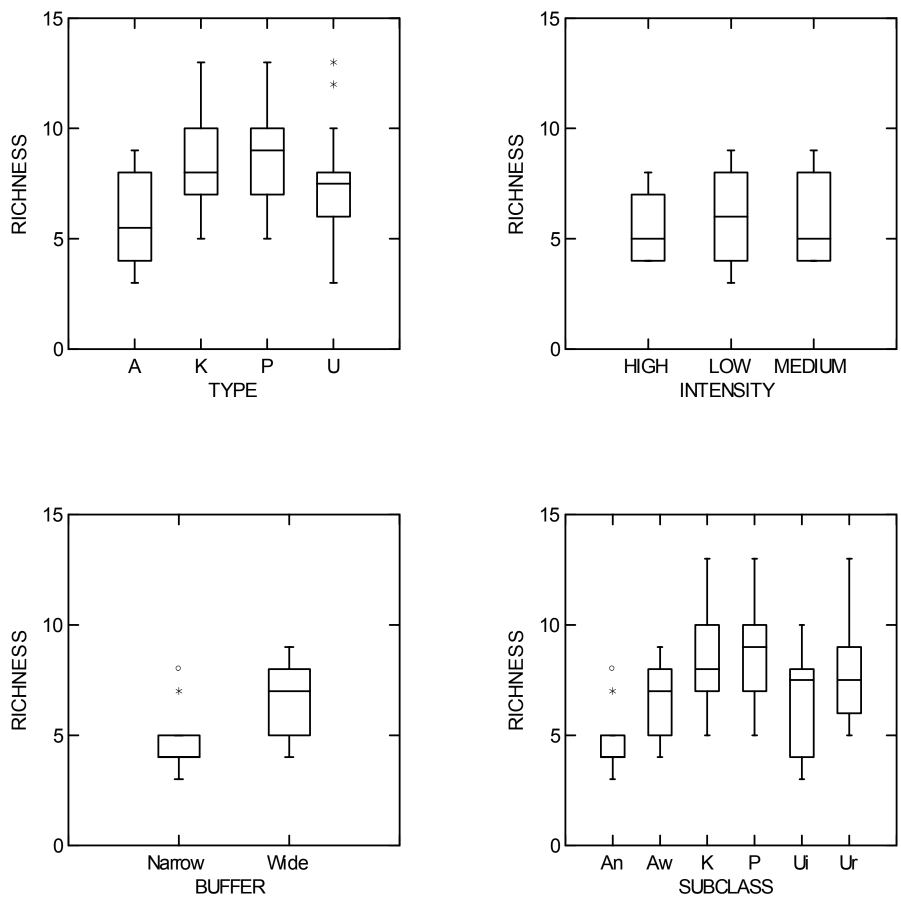
** excluded are 4 taxa shared only between kettles and prairies and one taxa shared between impacted classes; 15 taxa were distinct to combined reference classes and 10 taxa were distinct to impacted wetlands.

Some taxa that occurred in both reference and impacted wetlands occurred more frequently (Chi-squared analysis, df=1, $p<0.05$) in reference wetlands than in impacted wetlands. The most obvious examples included *Simocephalus vetulus* (occurred in 92% of reference wetlands: 29% impacted) and *Ceriodaphnia* spp. (94% reference: 74% impacted). Unfortunately, many of the taxa occurred too infrequently to meet the statistical testing requirements and therefore, their occurrences are of little indicator value.

Despite some apparent differences in the taxonomic composition among the wetland classes, extensive analysis of the data, using a combination of multivariate techniques and association analysis of community structure (Dodson & Lillie In Prep.), failed to reveal any associations with land use class. No distinct pattern was discovered among taxa occurrences, and co-occurrences were not evident.

Taxon richness ranged from 3 to 13 taxa per site (Appendix Z) and averaged 7.5 taxa (95%C.I.= 6.9-8.1; SE=0.3) across all sites. Taxon richness differed significantly among wetland classes (ANOVA, df=3, F-ratio=6.453, p=0.001; Fig. Z1). Taxon richness was significantly higher in kettle and prairie reference wetlands than in agriculturally impacted sites. Other pair-wise comparisons of taxon richness among the four classes (using posthoc tests) were not significantly different ($p>0.05$). Taxa richness did not differ significantly among the three intensity levels of agricultural impact (ANOVA, $p>0.05$), but buffer width evidently had a significant influence on taxon richness (t-test, df=18, pooled variance t= -2.263, p=0.036). Agriculturally impacted wetlands with narrow buffer strips had on the average 1.86 fewer (95%C.I. 0.13 to 3.58) taxa than counterpart wetlands with wide buffer strips. Taxon richness also varied according to a number of land cover variables discussed in detail in the paper in preparation (Dodson & Lillie In Prep.).

Figure Z1. Box plots of zooplankton taxonomic richness by wetland type, agricultural intensity, buffer class, and land use subclass.



The occurrence pattern of *Daphnia pulex* and percentage of sites with males varied among wetland classes (Table Z2). Although these differences were not statistically significant (Chi-squared analysis, all $p>0.05$), *Daphnia pulex* occurred at 81% of the reference sites but was only found at 55% of the agriculturally impacted sites and 50% of the urban-industrial sites. Interestingly, *D. pulex* occurred at all 10 urban-residential sites. Among sites where *D. pulex* occurred, males tended to occur more frequently at agriculturally impacted sites (40%-50%) and at urban-industrial sites (50%) than at reference sites (27% kettles and 29% prairies). Males occurred least frequently at urban-residential sites.

The combination of either the presence of male *D. pulex* or the absence of both male and female *D. pulex* may be a good indication of stress on the wetland. Within our data set, 70% of the agriculturally impacted wetlands experienced this condition as did 75% of the urban-industrial wetlands. Only 41% of the prairie reference wetlands and 42% of the kettle reference wetlands matched the criteria. Again, the urban-residential wetlands appeared to be under less stress, with only 20% of the sites fitting this condition.

Table Z2. Occurrence of *Daphnia pulex* by wetland category. Data excludes replicates.

Classification	N=	Females present	Males present	Females only	Both absent	Percent both absent or males present
Agriculture – narrow buffer	9	6	3	3	3	67%
Agriculture – wide buffer	11	5	2	3	6	73%
Reference – kettles	19	15*	4	11	4	42%
Reference – prairies	17	14	4	10	3	41%
Urban – industrial	8	4	2	2	4	75%
Urban – residential	10	10	2	8	0	20%
Totals	74	54*	17	37	20	50%

* excludes one reference kettle that contained a single *D. pulex* in replicate sampling.

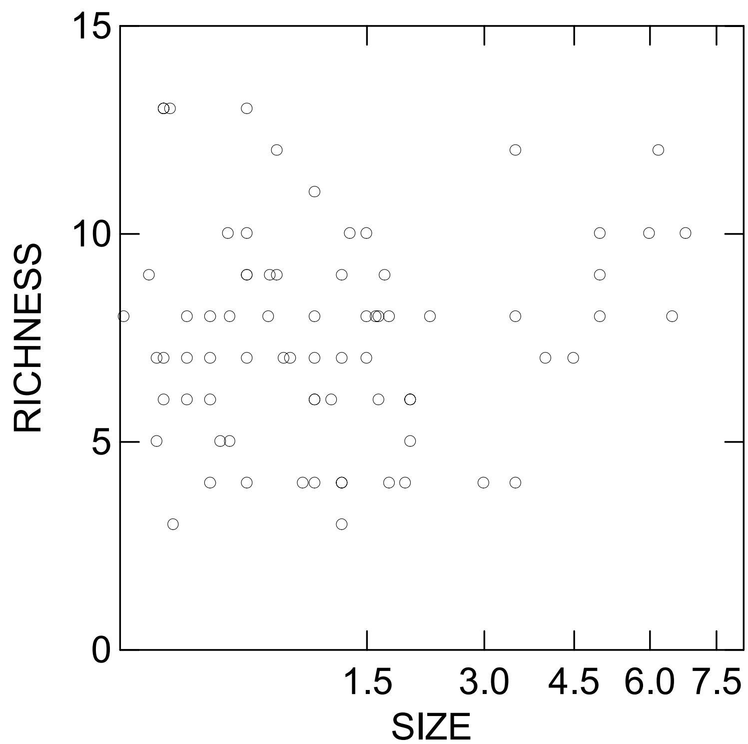
Development of Zooplankton Biotic Index

Three metrics were chosen for inclusion in a preliminary index of wetland condition based on the zooplankton community. The metrics include 1) total taxon richness, 2) the presence of male *Daphnia pulex* or absence of all *D. pulex*, and 3) an indicator metric weighted according to the relative frequency of occurrence of 10 taxa that tended to occur more frequently in reference wetlands than in impacted wetlands. The procedures and rationale used in scoring each metric are discussed in the following paragraphs.

Taxon Richness Metric:

Taxon richness differed among wetland classes and between buffer widths among the agriculturally impacted wetlands. We assigned metric scores based on the tri-sectioning of the 74 data points as follows: less than 2 taxa = 0, 2-5 taxa = 1, 6-9 taxa = 3, and greater than 9 taxa = 5. Some form of adjustment may be warranted in the future to account for an increase in taxa richness with the size of the wetland (see Fig. Z2). The sample size in the greater than 2 acres category was insufficient to do so at this time. The distribution of scores among the 74 wetlands was sixteen '1's, forty-three '3's, and fifteen '5's.

Figure Z2. Taxa richness versus wetland basin size (in acres).



The Indicator Metric:

The presence/absences of all 59 taxa were summarized according to their distribution in the 36 reference wetlands (kettles and prairies combined) and in the 38 impacted wetlands (agriculture and urban combined). In most cases taxa occurred either too infrequently to administer a valid chi-squared test (or Fisher-Exact test) or the taxa distributions were not significantly different. Among the 59 taxa, seven taxa produced significant results (Table Z3) and were included in a composite metric along with three additional taxa that were infrequent in occurrence but restricted to reference wetlands only.

Table Z3. Results of Chi-squared analysis of the distribution (presence/absence) of zooplankton taxa between reference wetlands and impacted wetlands. The d.f. was 1 for all comparisons.

Taxon	Chi-square value	p-value
Ceriodaphnia spp.	4.436	0.035
Simocephalus vetulus	27.62	<0.001
Mesocyclops americus	4.238	0.040
Diacyclops leptopus	6.513	0.011
Cyclocypris sp.	10.057	0.002
Microcyclops rubellus	5.136	0.023
Lynceus brachyurus	*	0.023

- Fisher-Exact test substituted for Chi-square analysis.

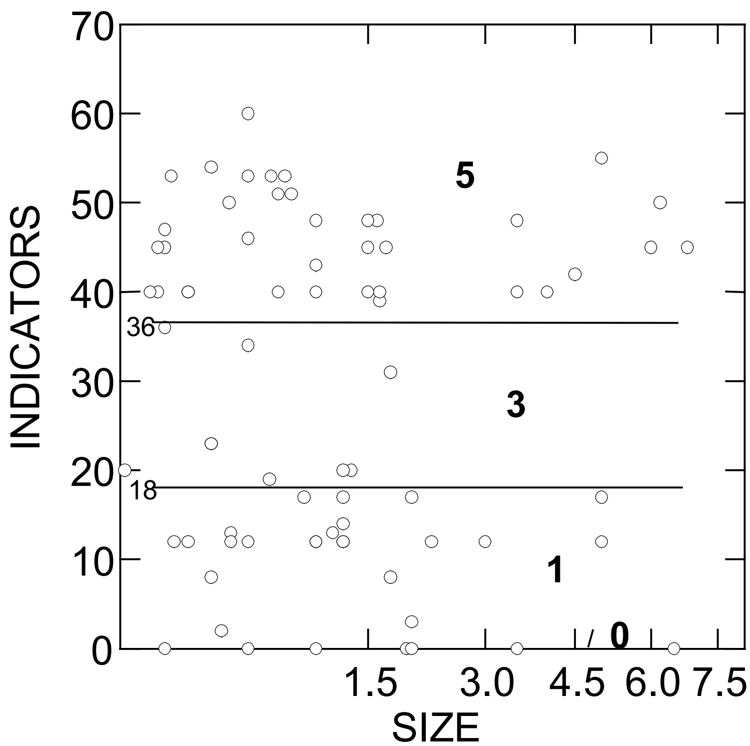
A composite indicator metric scoring system was developed based on the absolute difference in frequencies of occurrence for each taxon between reference and impacted wetlands multiplied by the total occurrences of the taxon in all 74 wetlands. This procedure essentially gives weight (i.e., higher scores) to a taxon based on the combination of its overall occurrence (common taxa more likely to score higher than rare taxa) and the quantitative disparity in distribution between reference (assumed to be relatively un-impacted) and impacted wetlands. As an example, *Simocephalus vetulus* occurred in 33 of 36 reference wetlands but occurred only in 11 of 38 impacted wetlands. The difference in frequencies of occurrence between reference and impacted wetlands was 0.628 (i.e., 0.917 – 0.289). To derive an indicator score for this taxon, we multiplied the difference by the total number of occurrences, or 44, to derive 27.632 (rounded to 28). We followed this procedure to assign scores for the other six taxa (Table Z4). Finally, we added three additional taxa that occurred very infrequently, but occurred only in reference wetlands. These three taxa, *Diacyclops nearcticus, Acanthocyclops vernalis*, and *Ostracod* sp. H, were assigned one point each. The composite indicator score for each wetland is the sum of each of the individual scores.

Table Z4. Assignment of scores for taxa included in the indicator metric.

Taxon	Indicator score
Simocephalus vetulus	28
Ceriodaphnia spp.	12
Cyclocypris sp.	8
Microcyclops rubellus	5
Lynceus brachyurus	5
Diacyclops leptopus	3
Mesocyclops americus	2
Diacyclops nearcticus	1
Acanthocyclops vernalis	1
Ostracod sp. H	1

The individual indictor scores ranged from 0 to 60 among wetlands. Indicator metric scores were plotted against depth and tri-sected to derive metric scores (Fig. Z3). Scoring was as follows: 0-17 = 1, 18-36 = 3, and > 36 = 5. The distribution of scores among the 74 wetlands included seven '0's, twenty-two '1's, eight '3's, and thirty-seven '5's.

Figure Z3. Metric scores for Indicator values plotted against wetland basin size.



The *Daphnia pulex* metric:

Daphnia pulex occurred slightly more frequently in reference wetlands than in wetlands in agricultural settings or urban-industrial settings. Where *D. pulex* was found, male *D. pulex* occurred more frequently in these same impacted wetlands. If the presence of males is evidence of some form of stress, then the absence of both males and females may be suggestive of extreme stress. While 75% of the wetlands in agricultural and urban-industrial settings either had male *D. pulex* present or *lacked D. pulex* altogether, less than half of the reference wetlands experienced such a condition. Based on the assumption that *D. pulex* was an indicator of environmental stress, we assigned metric scores to each wetland based on the combination of the presence of *D. pulex* and presence or absence of males as shown in Table Z5. Twenty wetlands received a score of '1', seventeen scored a '3', and thirty-seven rated a '5'.

Table Z5. Assignment of *Daphnia pulex* Metric scores.

Criterion *Daphnia pulex** Males	Females	Metric Score	Perceived Condition
Absent	Absent	'1'	Highly stressed?
Present	Present	'3'	Stressed
Absent	Present	'5'	Natural

* males were never found where females were absent.

<u>The Wetland Zooplankton Biotic Index</u>:

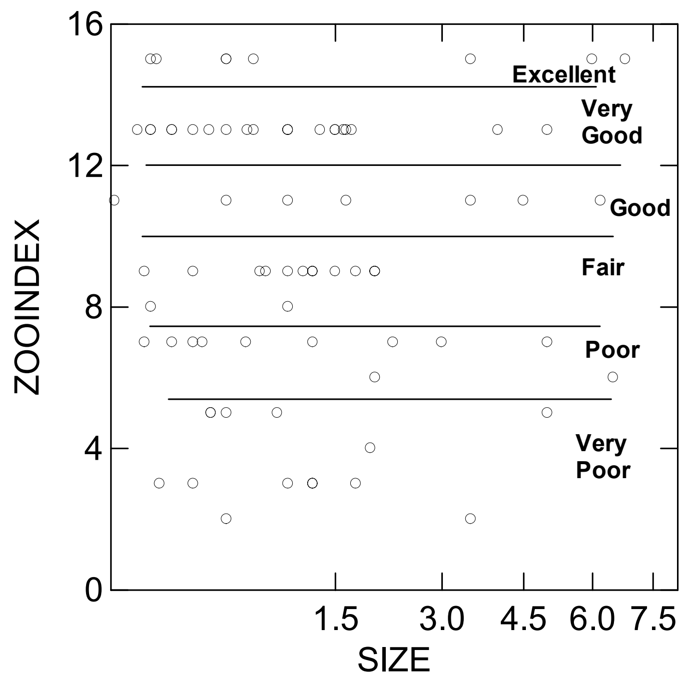
The zooplankton community index (Table Z6) was calculated by summing the three component metric scores. The range in values extends from a minimum of 2 to a maximum of 15 (each metric scored as a '5'). Zooplankton metric scores for individual wetlands are provided in Appendix Z. The median index value was 9 with a mean of 9.55 (95%C.I. = 8.66-10.45). It should be noted that because of the small number of metrics used in the index, it is not possible to receive a score of 12 or 14.

Table Z6. Assignment of scores for the Wisconsin Zooplankton Biotic Index.

Taxa	**Attribute**	**Limitations**	**Response to disturbance**	**Scores** **0**	**1**	**3**	**5**
All taxa	Richness	Wetland Size?	Decrease	0-2	3-5	6-9	∃10
10 indicator taxa	Presence (times Scores)	More taxa may be added	Decrease	no	0-18	19-35	∃36
Daphnia pulex	Presence & Absence	None	Males appear under moderate stress; both males + females disappear under heavy stress?	no	% = 0 & = 0	% > 0 & > 0	% = 0 & > 0

Qualitative ratings were assigned to the Zooplankton Biotic Index Scores roughly on the basis of distribution quartiles (Fig. Z4); the median of 9 was set as the threshold with the result that 47% of the scores received a rating of 'good' or better. Breakpoints were Excellent =15, Very Good = 13, Good = 10-11, Fair = 8-9, Poor = 6-7, and Very Poor < 6.

Figure Z4. Rating system for zooplankton biotic index scores.



Wetland Zooplankton Index Performance:

The discriminatory ability of the index was evaluated using ANOVA and Bonferroni post-hoc tests (results provided in Table Z7). Land use (at both type and subclass scales) had a significant effect on the index values, but agricultural intensity and buffer width did not. The zooplankton index did a good job in identifying agriculturally impacted wetlands (Fig. Z5). Seventy percent of the samples from agriculture sites had a rating of poor or very poor, while only 8% of the reference wetlands rated poor (none very poor). Likewise, the index was obviously sensitive to industrial pollutants as 75% of the urban industrial-commercial sites rated poor or very poor. However, the index was not sensitive to residential runoff as only two urban residential wetlands rated as poor.

Figure Z5. Box plots of zooplankton biotic index scores by wetland type, subclass, agricultural intensity, and buffer class.

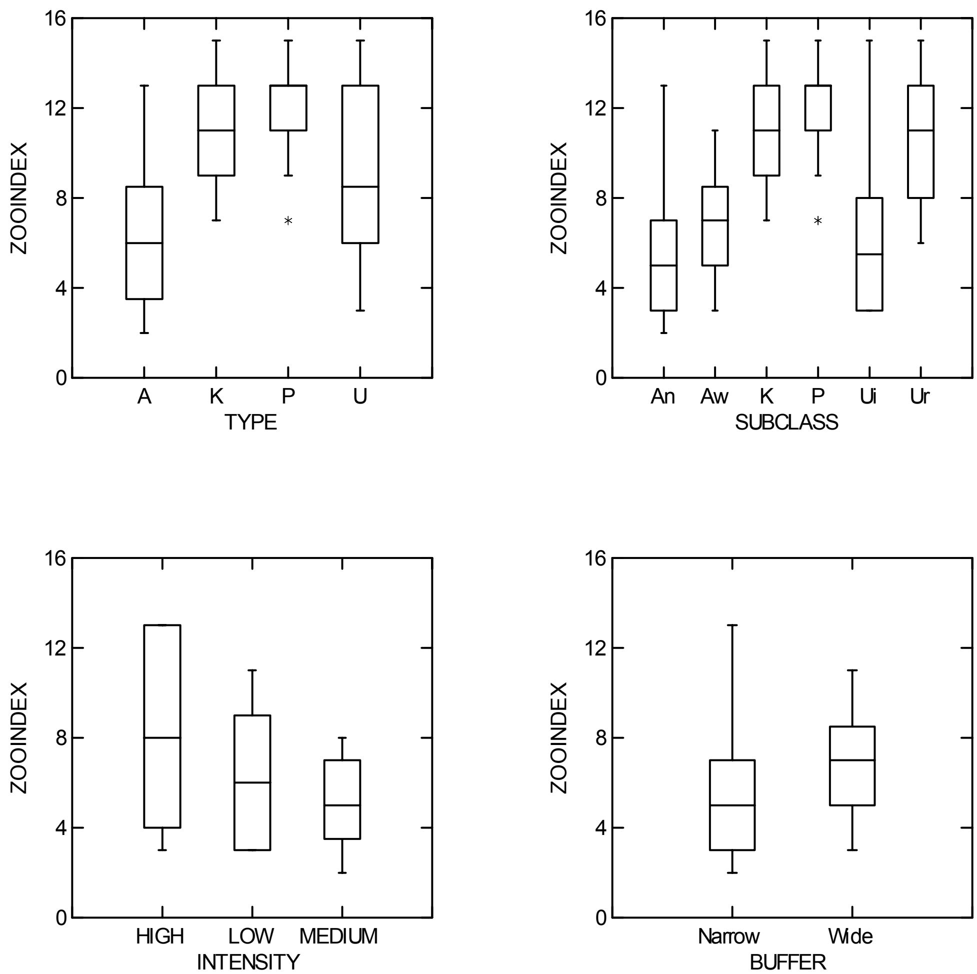


Table Z7. Results of significance testing of Zooplankton Biotic Index by selected wetland classifications.

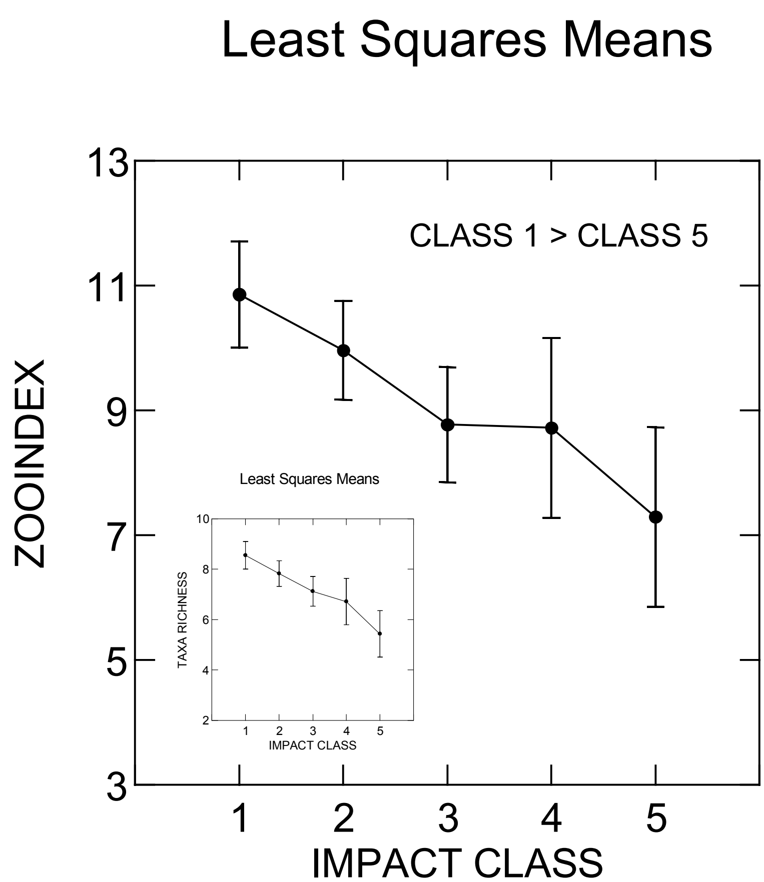
Anova By:	df	F-ratio Probability	Bonferroni significant comparisons:
Type	3	12.214 $p < 0.001$	K > A, P > A, P > U
Buffer	1	n.s.	not tested
Intensity	2	n.s.	not tested
Subclass	5	10.092 $p < 0.001$	K > An, K > Aw, K > Ui, P > An, P > Aw, P > Ui Ur > An, Ur > Aw
Predator Class	4	4.187 $p = 0.004$	C > F, N > F
Predator Class (N = 18* Agriculture only)	3	0.607 p = n.s.	All pair-wise comparisons n.s.; however, index values in N about 2 units higher than in wetlands with predators.

* excludes the two agricultural wetlands with salamanders present.

Anova analysis further suggested that the presence of predators (and type of predator present) in the wetlands had a significant influence on index values (Table Z7), but this finding was primarily an artifact resulting from the uneven distribution of predators among the various wetland types (or subclasses). Only one of the 19 reference kettles contained fish, while a much larger proportion of the agricultural (6 of 20) and urban wetlands (8 of 18) contained fish. Therefore the results of the anova tests across predator classes were highly influenced by the disproportionate number of reference wetlands in the ‘no-predators’ class (27 of 47) relative to the number of impacted wetlands in the ‘fish-present’ class (14 of 18). Rerunning the analyses using only the agricultural wetlands (6 with fish, 12 without fish) produced non-significant findings (ANOVA, df=3, F-ratio =0.607, p=0.620). Despite the negative findings, zooplankton index values in the agricultural wetlands without predators averaged 2 units higher than wetlands with predators. Conversely, among the eight urban industrial wetlands, the five wetlands with fish had higher zooplankton index values than the three wetlands without fish. Within the urban residential wetlands, the five wetlands without fish had index scores 4-6 units higher than three wetlands with fish, but unfortunately the sample size was inadequate and variability too great to produce statistically significant findings.

Index values were not significantly different among the five quantitative impact classes (ANOVA, df=4, F-ratio = 1.534, p=0.202) based on nutrient and chloride concentrations. However, the zooplankton biotic index values did decline progressively across the five impact classes (Fig. Z6). Zooplankton taxa richness declined significantly among the impact classes (ANOVA, df=4, F-ratio=2.584, p=0.045).

Figure Z6. Zooplankton index values (mean ± 95% CI) across wetland impact classifications. Only significant difference (p<0.05) was between class I and class V. The inset shows zooplankton taxa richness plotted versus impact class.



CORRESPONDENCE AMONG INDICES & RELATED ISSUES

The scales and ranges differed among the five community biotic indices (Table S1). The minimum and maximum scores possible for each index were a function of the number of component metrics in each index and the assignment of scores to individual metrics. The plant biotic index had the lowest amount of variation in scores.

Table S1. Descriptive characteristics of the biotic indices. N=74 each.

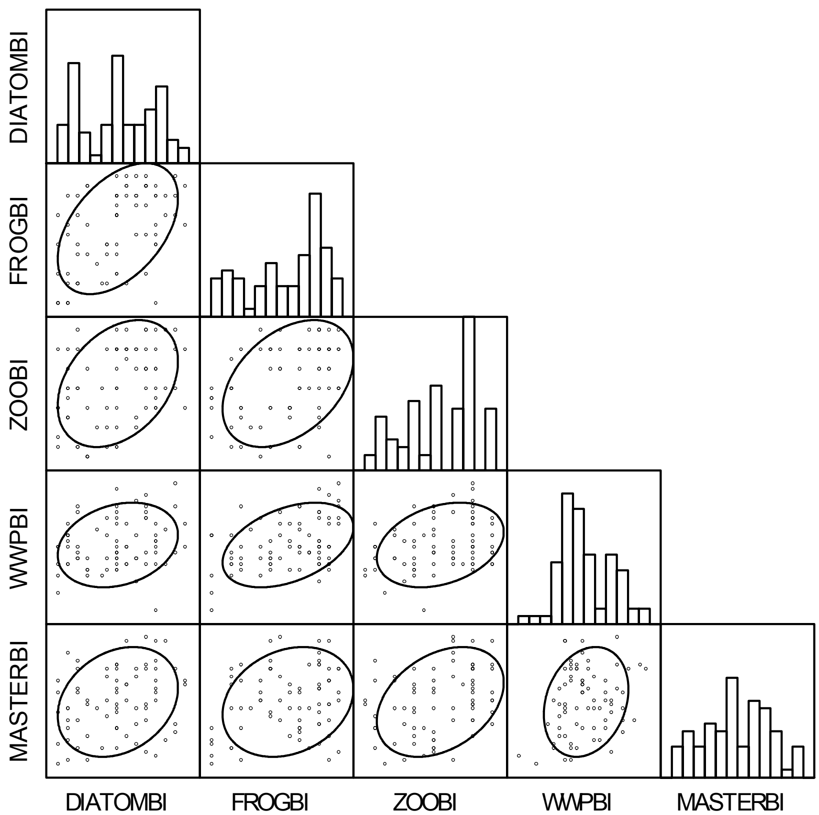
Attribute	Diatom BI	Frog BI	Zooplankton BI	Plant BI	Macroinvert-ebrate BI
Number of metrics		3	3	9	8
Minimum	9	0	2	4	3
Maximum	35	13	15	26	35
Median	21	9	9	15	19
Mean	21.0	7.7	9.6	15.5	18.6
95%CI Upper	22.7	8.6	10.5	16.5	20.7
95%CI Lower	19.4	6.8	8.7	14.5	17.0
S.E.	0.847	0.460	0.446	0.504	0.951
CV %	34.6	51.3	40.1	28.0	43.4

Scores among the five indices were strongly correlated with each other (Table S2 and Figure S1). The correlation between the macroinvertebrate and plant biotic indices was the weakest, yet statistically significant.

Table S2. Correlation matrix among biological indices.

Community Biotic Index	**Diatom BI**	**Frog BI**	**Zooplankton BI**	**Plant BI**	**Macroinvert. BI**
Diatom BI	XXXX				
Frog BI	0.463 $p<0.001$	XXXX			
Zooplankton BI	0.350 $p=0.002$	0.399 $p<0.001$	XXXX		
Plant BI	0.241 $p=0.039$	0.416 $p<0.001$	0.307 $p=0.008$	XXXX	
Macroinvert-ebrate BI	0.255 $p=0.028$	0.232 $p=0.047$	0.352 $p=0.002$	0.230 $p=0.049$	XXXX

Figure S1. Plots of correlations among the biotic indices. Macroinvertebrate biotic index labeled as MASTERBI and plant biotic index is labeled WWPBI.



The response of each index to watershed type, land use subclass, agricultural intensity, buffer width class, and impact category is compared in the form of a series of box plots in figures S2-S6. All indices were sensitive (i.e., statistically significant differences were detected) to watershed type (Fig. S2), land use sub class (Fig. S3), and impact category (S6) (albeit zooplankton BI only separated the two extreme classes). Both the plant and diatom BI indices tended to score prairie reference wetlands lower than counterpart reference kettles (Figs. S2 and S3).

None of the biotic indices showed statistically significant differences among the three levels of agricultural intensity (Fig. S4). This apparent inability of the indices to show a statistically significant difference among the three subjective levels of agricultural intensity may indicate that most of the damage (i.e., impact) to the community structure may be done at a much smaller level. For example, perhaps 10% or 25% agricultural development in the watershed is sufficient to cause a significant decline in some of the more sensitive communities. The macroinvertebrate BI and frog BI suggested a continuing decline in the moderate to heavy intensity levels.

The Diatom BI was the only index to successfully separate the impact of buffer classes (Fig. S5). Application of general linear model analysis (Systat 1996) failed to reveal any interactions between intensity and buffer. It is likely that the small sample sizes and the use of the average width of the buffer zone (rather than some other measure of the effectiveness of a crop buffer) contributed to the findings. Zooplankton richness also was responsive to buffer class, but the zooplankton bitoic index was not.

Plots of bitoic index scores versus individual environmental attributes (plots not presented here) were used to determine the sensitivity of the respective indices. Macroinvertebrates appeared to be most sensitive to total nutrient concentrations, while the frog and diatom indices were particularly sensitive to chloride concentrations. These findings were readily apparent in the responses among impact classes (see Fig. S6).

Figure S2. Box plots of five biotic indices by wetland watershed typology.

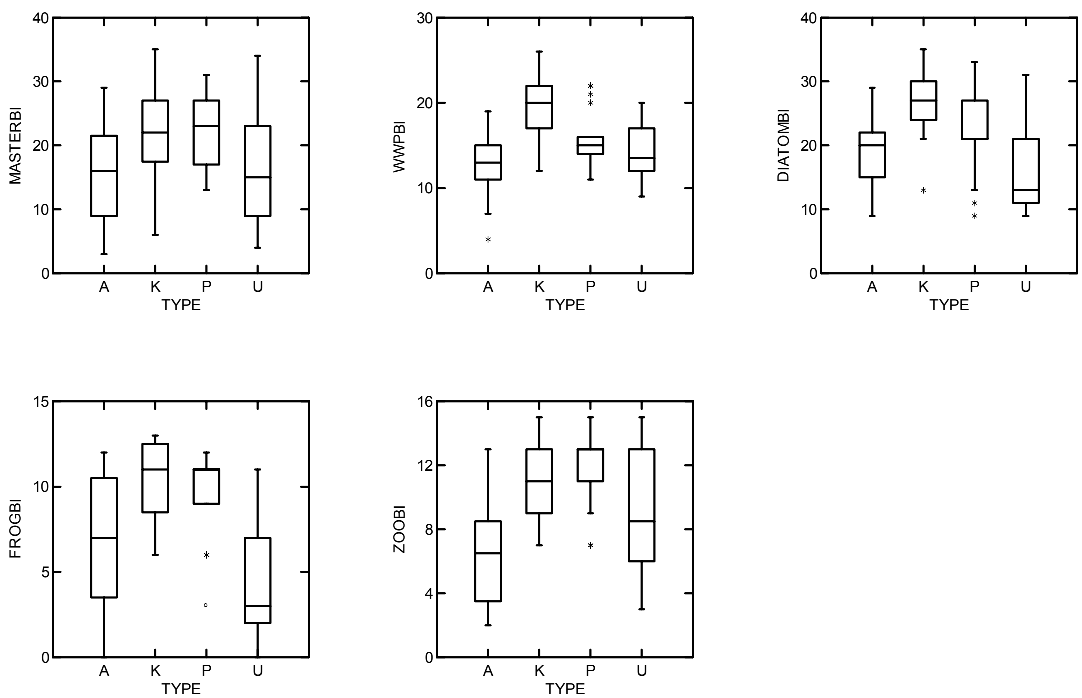


Figure S3. Box plots of five biotic indices by wetland land use sub-class.

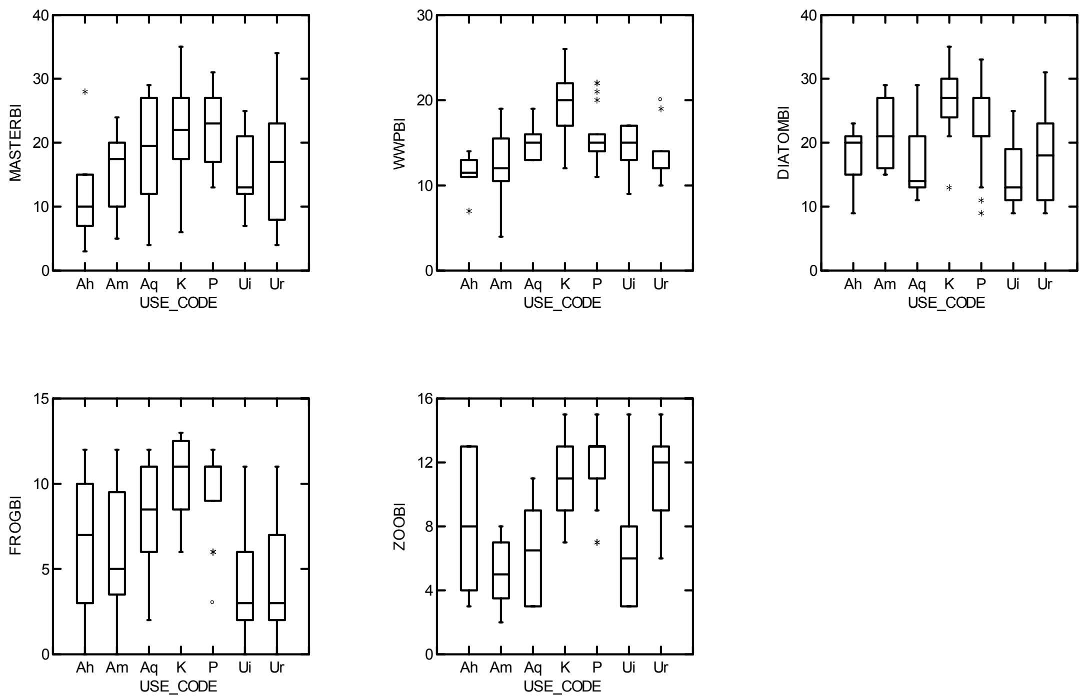


Figure S4. Box plots of five biotic index scores by agricultural intensity level. N=20.

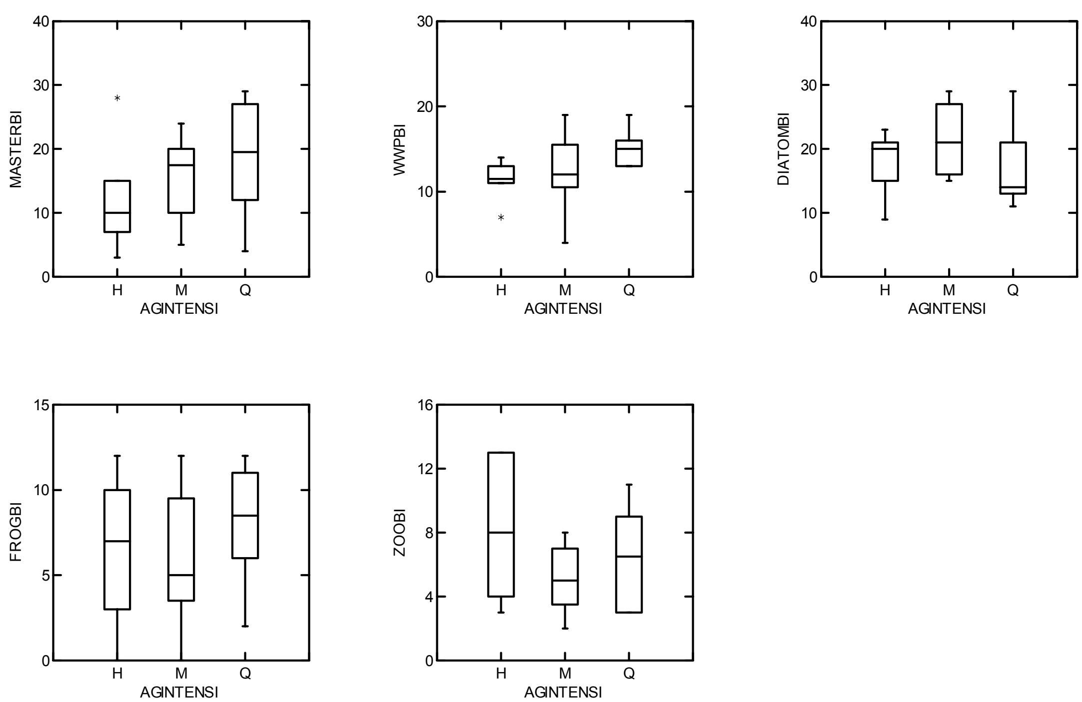


Figure S5. Box plots of five biotic indices by buffer width classification. N =narrow < 10 meters and W = wide > 10 meter average buffer width.

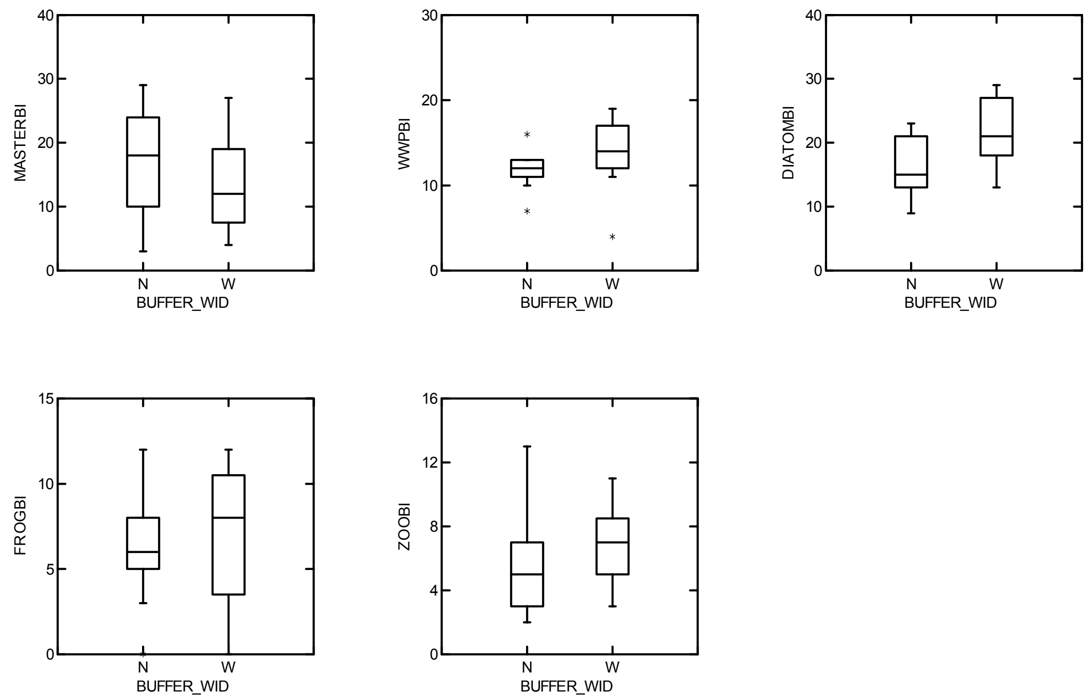
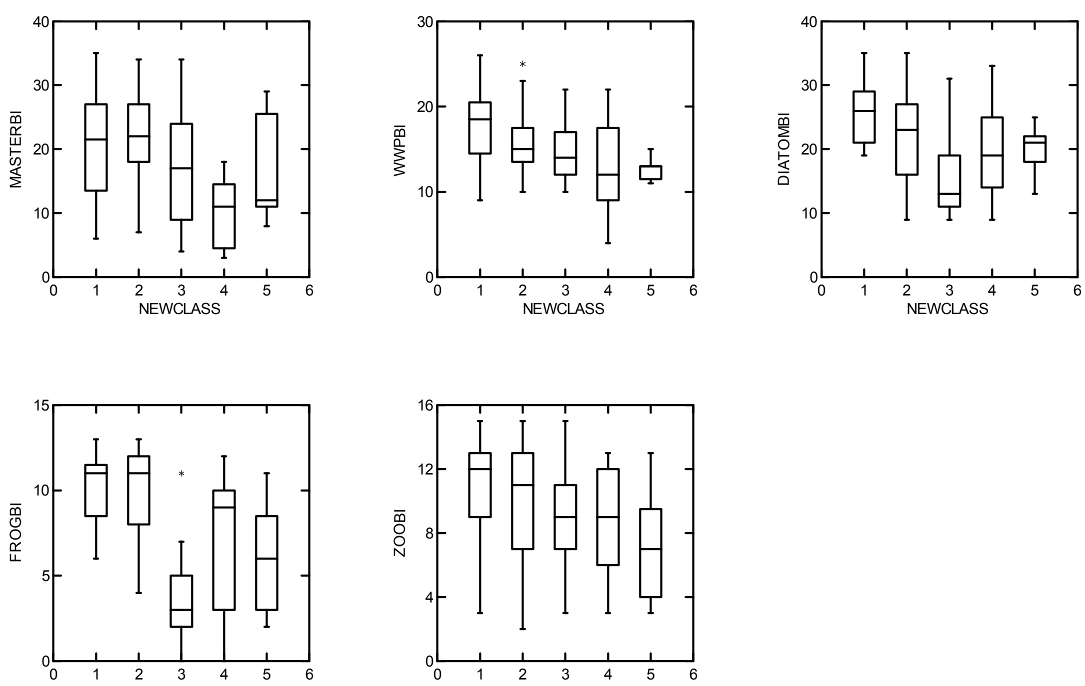


Figure S6. Box plots of five biotic indices by impact classification. Classes coded as follows: Class I = low-low chlorides and nutrients, Class II = low to moderate chlorides or nutrients, Class III = high chlorides, Class IV = High nutrients, Class V = high chlorides and high nutrients.

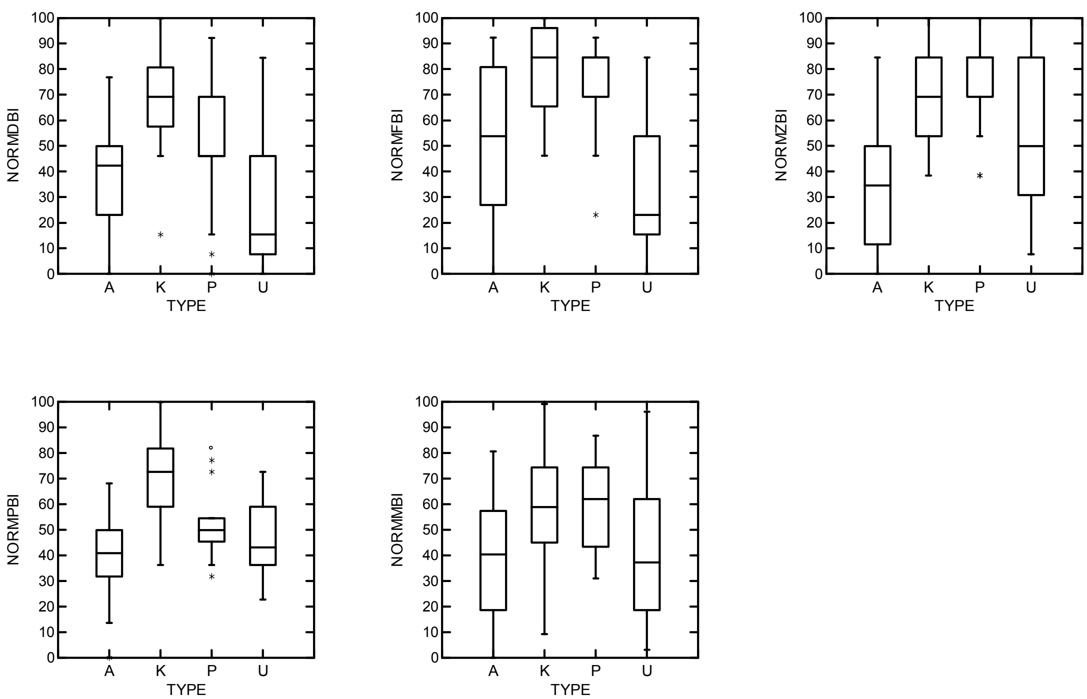


Index of Ecological Integrity

We explored several options for developing an index of ecological integrity in small depressional wetlands using the five biotic indices developed in this study. The options included 1) adding the component biotic scores, 2) averaging the component biotic scores, and 3) selecting the minimum score among the five biotic indices. Option one is the simplest and most straight-forward approach, but information is lost regarding variability. The second option produces essentially the same result as the first, but reduces the scale of scores. However, it also offers a possible advantage in permitting the reporting of some estimate of variance (e.g., the standard deviation of the five component index scores). The third approach was accomplished under the assumption that one (or more) community might be more sensitive to human impact than the other five communities evaluated in this study. After testing and evaluating the sensitivity of the three approaches, we adopted the averaging method.

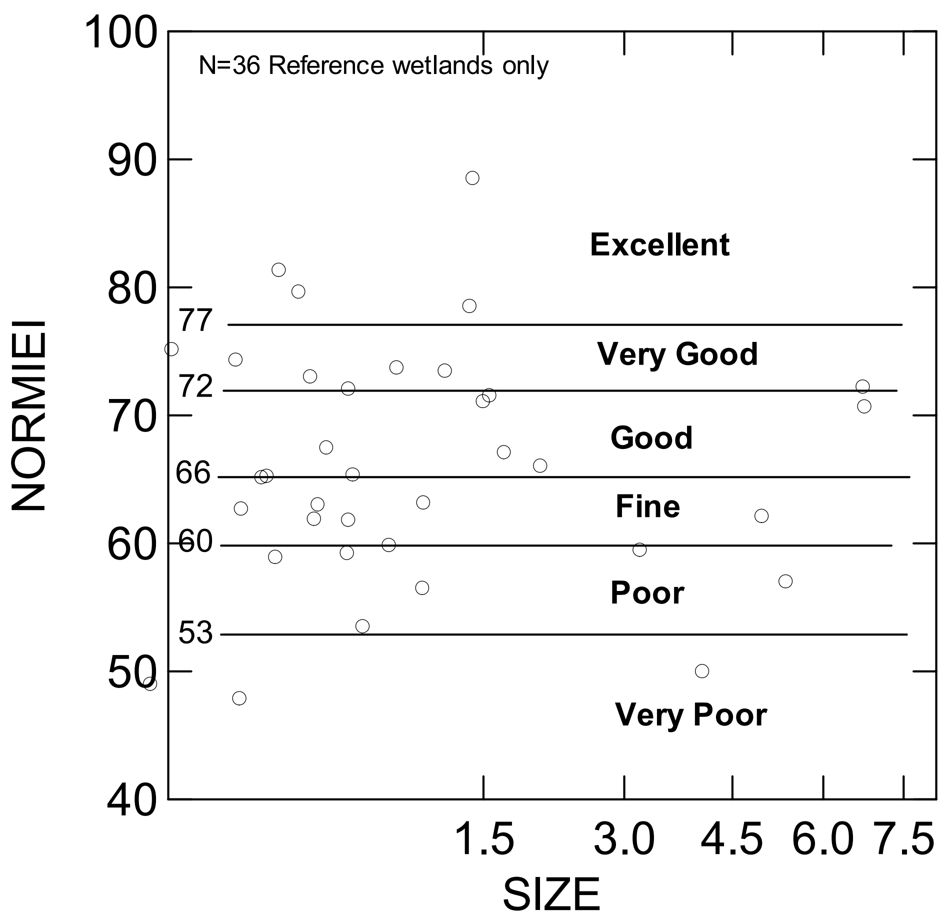
Prior to integrating the component metrics into a composite index, we normalized each individual biotic index score to adjust for the scaling differences among the indices. For each index, we subtracted the minimum observed value from each of the other scores and multiplied the resulting score by a variable that would produce a maximum score of 100 for each index. This transformation essentially gives equal weight to each biotic index without influencing the discriminatory power of the index (e.g., see Fig. S7 below).

Figure S7. Response of normalized biotic indices to wetland type. Diatoms = NORMDBI, Frogs = NORMFBI, Zooplankton = NORMZBI, Plants = NORMPBI, and macroinvertebrates = NORMMBI.



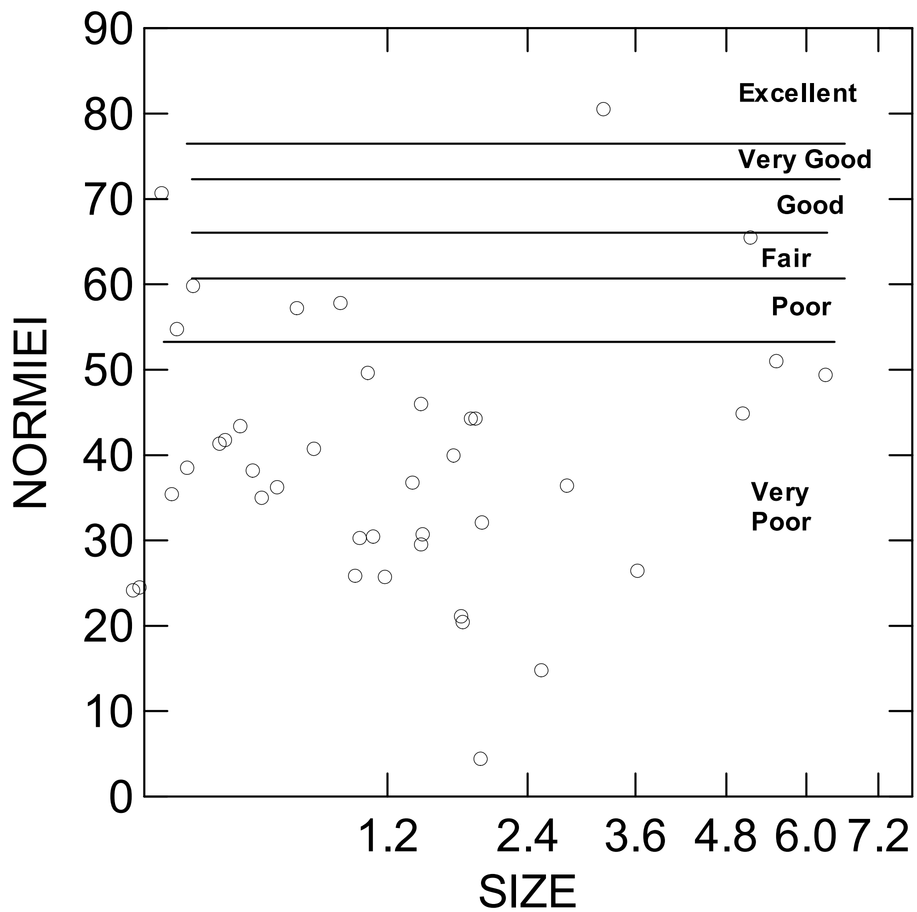
The wetland index of ecological integrity (IEI) was calculated as the average of the individual normalized biotic index scores for each wetland. We developed a qualitative rating system for the index based on the distribution of the scores among the 36 reference wetlands (Fig. S8). The median and mean IEI scores were 66; 95%CI = 63-70; SE = 1.56; CV = 14.1%; and range from 47.6 to 90.2. By our definition, the breakpoints for separating the qualitative ratings were set at the 10, 25, 50, 75, and 90 percentiles.

Figure S8. Qualitative rating system for index of ecological integrity.



Computation of IEI values for sites classified as urban or agriculturally impacted shows that 79% of the sites rate as ‘Poor’ using the qualitative rating system (Fig. S9). Only a few minimally impacted sites rated as well as the majority of the reference sites.

Figure S9. Scores for Index of ecological integrity among urban and agriculturally impacted wetlands. N = 38.



The degree to which the IEI represents the ecological integrity of an individual wetland was judged on the basis of the agreement (or lack thereof) among the five component biotic index scores. This value was calculated as the standard error of the five component scores (post normalization). The median deviation for the 74 wetlands was 22.2 IEIs, ranging from a minimum of 8.0 to a maximum of 35.6 IEI units. The average deviation was 21.7 IEIs with 95%CI = 20.2-23.2 and a SE = 0.76.

We suspected that the variation among component biotic index scores might be greater in impacted wetlands than the reference wetlands, but this was not the case. No statistically significant differences in the standard deviation were found among wetland types, land uses, agricultural intensity levels, buffer classes, or impact classifications (ANOVAs). Standard deviations were highest within Class IV (high nutrients) and lowest within Class I (low chlorides and low nutrients), but the differences were not significant (p>0.05). There was also no pattern or trend detected in the magnitude of the standard deviation across the range of the individual biotic index values. The only exception was the hint of a slight increase in standard deviations with increasing plant biotic index scores, but the biological meaning of this response was not clear.

Consequently (in concert with examination of biplots between the various biotic indices), we concluded that there appears to be no consistent bias or pattern in the individual responses to suspected impacts among the five communities. In some wetlands, one community is affected, while in another wetland a different community may be affected. Therefore, while a high standard deviation is by itself is not very unusual, an IEI with a high standard deviation may signal that a closer examination of the data for that particular wetland is warranted. Determining which community (or communities) among the five communities examined may be responding 'differently' than the others may reveal clues as to possible causes for the observation.

Performance of Index of Ecological Integrity:

The IEI performed better (i.e., greater number of significant differences detected) than each of the individual biotic indices in detecting significant differences among wetland watershed classes and sub-classes (Table S1 and Fig. S10). It did not distinguish differences among levels of agricultural intensity nor between narrow and wide buffers. The IEI clearly separated the high nutrient and high chloride impact classes from the low to moderate impact classes (Fig. S11 and Table S1).

Table S1. Results of ANOVA analysis of the Index of Ecological Integrity among wetland type, land use sub-class, and impact classification. Analysis with agricultural intensity and buffer class were not significant (p>0.05).

ANOVA By:	d.f.	F-ratio	Probability	Fisher LSD post-hoc comparisons
TYPE	3	27.003	P <0.001	K > A, K > U P > A, P > U
Subclass	6	14.965	P <0.001	K > Aq, Am, Ah, Ui, Ur P > Aq, Am, Ah, Ui, Ur Ur > Ui
Impact Class	4	12.078	P < 0.001	Low > Both, Chlorides, Nutrients Med. > Both, Chlorides, Nutreints

Figure S10. Box plots of Index of Ecological Integrity by wetland typology, sub-class, agricultural intensity level, and buffer width class.

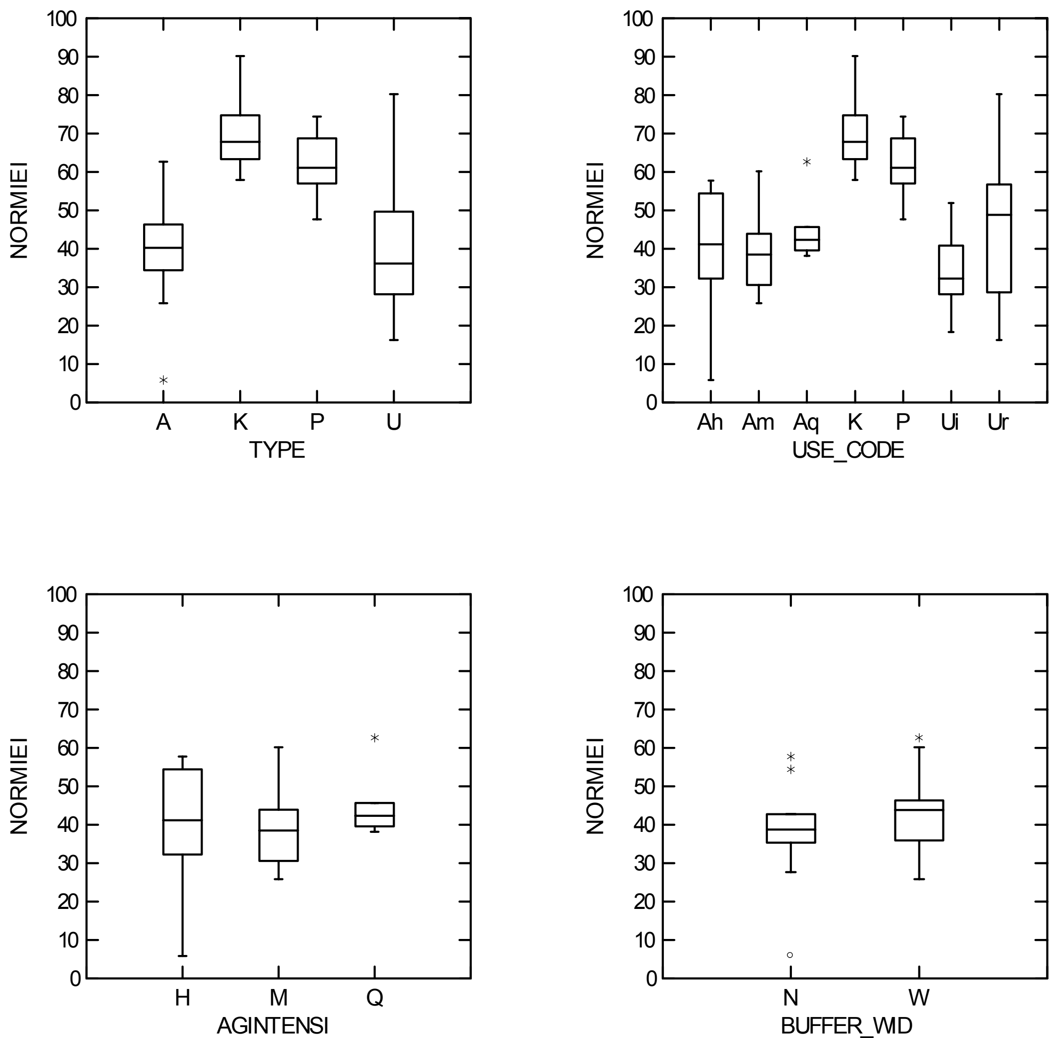
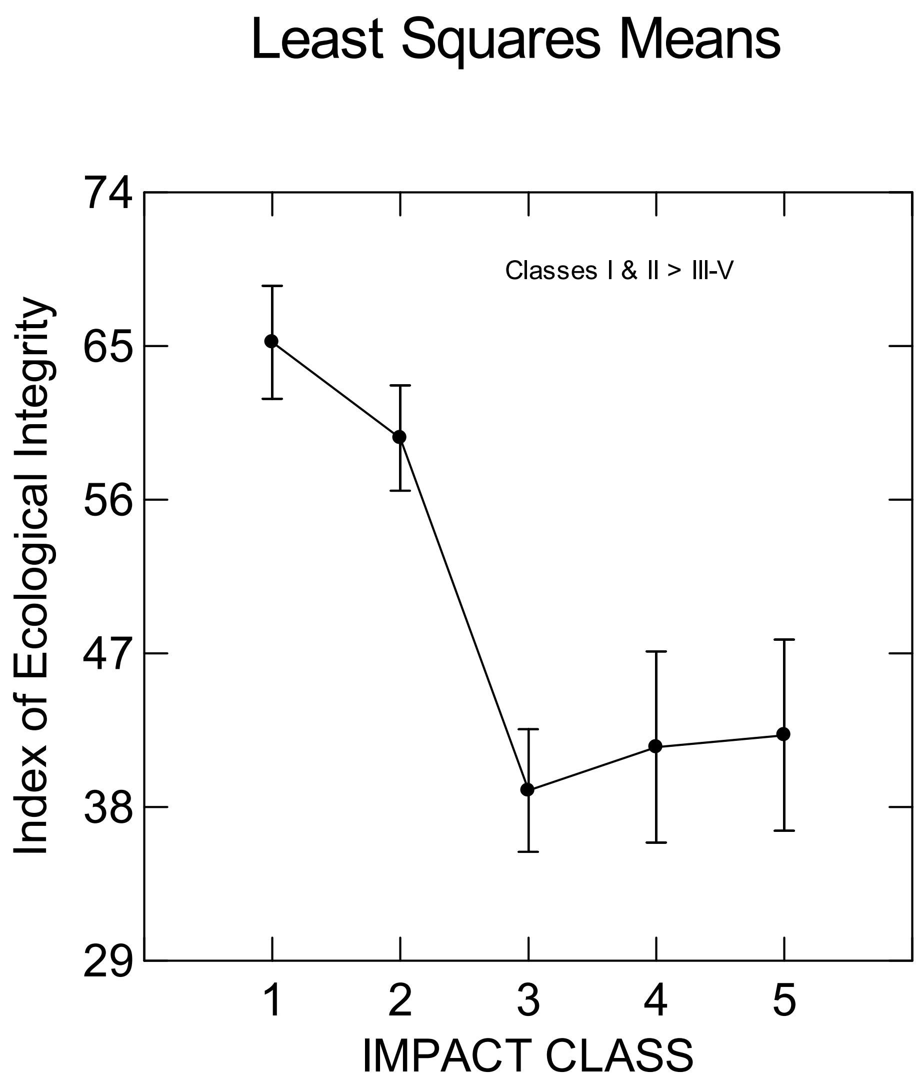


Figure S11. Differences in the mean Index of Ecological Integrity among the quantitative impact classes. Classes I and II represent the low to moderate concentrations of chlorides and nutrients; Class III represents high chlorides, Class IV represents high nutrients, and Class V represents high chlorides and high nutrients.



MANAGEMENT CONSIDERATIONS & CONCLUSIONS

The practical application of the methods for wetland bioassessment presented in this report remains to be tested. While the integrated Index of Ecological Integrity clearly did a better job in differentiating impact than each of the component biotic indices, it is unlikely that a management agency can afford the time and labor necessary to assess all five biological communities involved. Considering the fact that each of the component biotic indices functioned satisfactorily in the capacity to detect certain forms of human impact, it appears that the choice of which communities to assess is entirely up to the agency involved.

Table X1 presents a listing of the some advantages and disadvantages of the six biological communities evaluated as potential indices of wetland condition in this study. Although we were not able to identify any useful metrics using the small mammal community, it is possible that a more extensive effort will reveal some response indices in the future. The diatom and zooplankton indices require highly trained experts to identify the various taxa involved. This potential problem might be resolved by establishing a contract to provide such services with the State Laboratory of Hygiene or other independent contracting services. The frog biotic index probably is the easiest to apply, at least in regards to level of expertise. However, it does require two separate field visits. The plant biotic index may be surpassed by the development and future application of the floristic quality index (Swink & Wilhelm 1994, Herman, et al. 1997, and Minc & Albert 1998), which is currently being modified for use in Wisconsin (pers. com. T. Bernthal, WDNR). However, the promise of the plant biotic index is that the level of taxonomy involved does not require a trained botanist. Management staff (or public) could assess and calculate a plant biotic index value for a given wetland within a short time without involving a botanist and still achieve a useful index value. The macroinvertebrate index requires a considerable greater amount of effort, but may also be applied by non-entomologists with a minimal amount of training. Current evaluations are ongoing to evaluate the repeatability and accuracy of MBIs calculated by professional and non-professional staff.

Table X1. The advantages and disadvantages of monitoring selected biological communities.

Community	Advantages	Disadvantages
Macroinvertebrates	Minimal field collection effort- 30 minutes; coarse taxonomic level permits possible application by public; 24-hr turn-around possible.	Seasonal differences – best in early spring; 2-hr lab work-up required at minimum X3 magnification; Annual changes probable – variability yet undetermined. Specialized field equipment required – net & preservative. Least sensitive of the five indices tested!
Wetland plants	1-hr field survey; coarse taxonomic level permits application by public; results possible within hours if needed. May be used even if wetland is dry at time of visit.	Many taxa within similar groups may have different tolerances; misidentifications likely. Highly subjective (yet seems to work!). The relatively low scores in prairie reference wetlands may reflect lack of full recovery from historic disturbance.
Diatoms	Easy and fast to sample; by examining cores it is possible to reconstruct history of wetland. Also possible to sample when dry.	Expert required for laboratory identifications.
Zooplankton	Simple field collection effort; summer sampling.	Expert required for laboratory identifications. Introduction of predators in the system may influence index values?
Amphibians	Minimal amount of training required to learn frog-calls; ‘fun’ for public. Easy to calculate metrics.	Two field visits required; limited to spring-time only. Most sensitive to chloride impacts.
Small mammals	Incorporates riparian condition; ‘fun’? or highly visible for public.	2-days fieldwork; high trapping skills required; few taxa involved, difficult to identify; no metrics discovered.

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